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THE CORRELATION EFFECT FOR A HISTIDINE LOCUS OF NEUROSPORA CRASSA[1]

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Received January 28, 1957

FOR different organisms rare wild type segregants have been observed in crosses involving independently isolated mutants which all show the same phenotypic effect. In all cases in which markers on one or both sides of these mutational sites were present the segregants which are wild type with respect to the isolocal[3] markers are associated with outside marker combinations which in the crossing over theory would be assigned to 1, 2, and even 3 crossovers between and outside the isolocal markers. The correlation between these "recombinations" is so high that the occurrence of wild type segregants cannot be explained by statistically independent crossovers. The first report of this kind was given by DEMEREC (1928) who described the phenomenon as reverse mutation associated with crossing over in heterozygotes of *Drosophila virilis*. That neither statistically independent crossovers nor clusters of (reciprocal) crossovers can explain the results has most clearly been shown for *Neurospora crassa* by M. B. MITCHELL (1955 a,b), who found that of four asci containing a wild type segregant for the isolocal markers none contained the reciprocal double mutant. Further results have been published for Neurospora by GILES (1955), ST. LAWRENCE (1956), and DE SERRES (1956), for *Aspergillus nidulans* by PRITCHARD (1955) and for phages by STREISINGER and FRANKLIN (1956). Irregular asci have also been found in yeast by LINDEGREN (1955) and ROMAN (1956).

In this situation several points of view can be or have been suggested. Agreement among all workers in the field seems now to exist for the statement that there occur regions of "intimate" (or "effective") pairing statistically distributed along the chromosome. In these regions events take place which result in the irregular segregation for the isolocal markers and the correlated appearance of the described 1, 2 and 3 fold "recombinants".

Concerning the events within such a region of intimate pairing two different attitudes to the problem can be stated.

The conversion theories

On the one hand one may use the (unproven) assumption of exactly reciprocal material exchanges (after duplication) as cause for the recombinations between more

[1] This work was supported by the International Cooperation Administration and by the Deutsche Forschungsgemeinschaft.

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[3] We shall call different mutations *isolocal* if they cause the same phenotypic effect with respect to a given (for instance biochemical) test and if they are confined to the same small genetic region. This definition merely comprises the preliminary biochemical and genetic results which ordinarily are easy to obtain without referring to a particular model. It leaves open to further experimental specification whether the different mutations are identical or different, whether they give wild type segregants in two factor crosses or not, and whether they belong to the same cistron (BENZER 1956) or to different ones.

distant loci and suppose that the correlation effect is due to an additional phenomenon being observable only for isolocal or very close markers. This additional effect shall be called "*conversion*". A conversion, as here defined, could either be due to a "*directed mutation*" (LINDEGREN 1955), that is, a mutation of one strand under the influence of the homologous strand during pairing, or due to a *transfer*, that is, the exact duplication of a chromosomal piece with insertion into the homologous strand (MITCHELL 1955 a,b). In order to explain the frequent appearance of "3 fold recombinants" one has to assume, then, a high correlation between the occurrence of both events, the conversion and the reciprocal exchange. This correlation is supposed to come about because the two events have a high chance to occur simultaneously within the same region of intimate pairing.

Two alternatives may be considered concerning the chromosomal places at which the reciprocal material exchanges between any two of the four chromatids occur: In the *classical picture* exchanges can only occur *between* the biochemically functional units (genes). Therefore different occurrences (alleles) of the same gene can never recombine by such exchanges and wild type segregants can only be obtained by a directed back mutation. In contrast, a modern viewpoint supposes the gene to be a linear structure of a certain length within which exchanges can occur, these exchanges being more or less equally probable for each place of this chromosome region. This concept was strengthened by the results of BENZER (1955) who showed for *phages* that a large number of isolocal mutations belonging to the same cistron[3] are separable by recombination, and can, in fact, be arranged in a linear map. For other organisms a linear structure of the gene has not been proven, although the complexity of adenine loci in *Neurospora crassa* (DE SERRES 1956) as well as in yeast (ROMAN 1956) are indications in this direction.

There are thus two ways within the conversion model in which wild type segregants can arise in crosses involving two different isolocal mutants. Either a conversion can give rise to the unreciprocal appearance of a wild type allele, or an exchange between the two isolocal markers (if the mutations occurred at different places of a linear map) causes the combined appearance of a wild type and a double mutant recombinant.—In the classical picture the results of crosses involving two isolocal mutants should usually show a different behavior according to whether the isolocal markers belong to the same or to two different genes. When they belong to the same gene only directed backmutations could give rise to wild type segregants and without further exhanges in the marked region the parental type of outside markers would be obtained. The frequent appearance of the recombinant type of outside markers can be understood only when both a directed backmutation and an exchange on the right or the left of the isolocal markers often occur together within the same region of intimate pairing. See figure 1. Because we might expect the exchanges to occur with about equal frequency on the right or the left side of the isolocal markers, the two recombinant types of outside markers should be about equally frequent. An order of the isolocal markers could then not be established. When the two isolocal markers belong to two different genes, reciprocal exchanges could also occur *between* the two markers. One recombinant type would therefore be more frequent than the other, thus allowing one to arrange the markers in linear order.

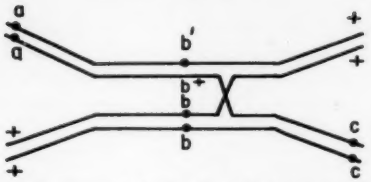

FIGURE 1.—Production of the type ab+c in the classical picture of the directed mutation hypothesis by a "directed backmutation" of the b′ allele and an additional reciprocal exchange.

If, in contrast to the classical picture, the "modern" standpoint is applied, in addition to conversions of one mutant, reciprocal exchanges would be expected to occur in crosses with nonidentical (or overlapping) isolocal markers, not only to the right or left of the markers but also between them, irrespective of whether they belong to the same gene or not. For marker distances for which the two mechanisms occurred with comparable frequency they could be detected in the same cross. However often one of the two limiting cases would be approached: For distant markers the classical effect of reciprocal exchanges would be predominant while on the contrary for very close markers the frequency of conversions (or directed mutation) could outweigh the occurrence of exchanges between the markers, resulting in an equal frequency of both recombinant types of outside markers.

The experimental evidence does not yet allow a decision. The 4-factor crosses of MITCHELL (1955 a,b) and GILES (1955) show in combination with wild type for the isolocal markers very unequal frequencies for the two recombinant types of outside markers. These results suggest in the conversion model that, at least for these markers, reciprocal exchanges can occur not only outside but also between the two isolocal markers. Yet these results cannot be taken as proof for the modern viewpoint because it has not been proven that the two markers in MITCHELL's or GILES' case belong to the same gene. Another set of crosses has been reported by ST. LAWRENCE (1956) in some of which the two recombinant types of outside markers occur nearly equally frequent (excepting the crosses with the seemingly aberrant q^4 marker). Such results cannot be regarded as a proof for the classical picture either, because the two isolocal markers might be so close that exchanges between them are much rarer than a conversion of one of them.

We conclude that the correlation effect can be explained by assuming that within regions of intimate pairing the mechanisms of conversion occur *in addition to* reciprocal material exhanges between or within genes.

A generalized crossover (switch) theory

On the other hand, one may prefer an interpretation of the correlation effect in which the events taking place within a region of intimate pairing do not involve two distinct but only one mechanism. This attitude is influenced by recent observations with phages revealing a strong correlation between the appearance of 1, 2 and 3 fold "recombinants" within short regions of the genetic map (STREISINGER and FRANKLIN 1956; DOERMANN, CHASE and EDGAR, personal communication). On the one hand, it seems impossible to explain these results by statistically independent exchanges

alone or by conversions alone. On the other hand, it appears rather improbable that two different mechanisms, such as exchanges *and* conversion, should both be involved in the production of three-fold "recombinants" for such a simple DNA structure. In attempting the construction of a unified theory, we can also use the results in phages (BENZER 1955) which suggest that a recombination between two markers, belonging to the same cistron, can occur in a large number of places between the markers. In order to explain the correlated occurrence of 1, 2 and 3 fold "recombinants" and the irregular segregation by just one mechanism we are led to an hypothesis which we will call the *switch hypothesis* and formulate as follows:

Within a region of intimate pairing two new chromosomal strands duplicate partially along one parental strand, partially along the other, switching forth and back from one information source to the other. The points at which switching can occur are continuously distributed along the genome. In other words, the probability that a switch occurs within a region of intimate pairing is approximately the same for each point within the region.

Because of the observed unreciprocal production of "recombinants" (MITCHELL 1955 a,b) we suppose that switching is often unreciprocal within small dimensions, although markers outside the switch area segregate reciprocally. Because of the observed correlation effect we suppose that several switches (at least 3) may occur within a region of intimate pairing; the average number of them is yet undetermined.

To illustrate these notions for a specific case, consider the cross in figure 2. In such a cross an ascus may arise containing two P1, one P2 and one irregular segregant like P2 except that it is wild type for *b* and *b'*. (For example, ascus 2 and 4 in MITCHELL's (1955a) case.) With the switch hypothesis this could be interpreted as seen in figure 3: One chromatid duplicates along one parent and because of the close pairing switches over to the other parent duplicating along there for a while (or incorporating an already duplicated piece) and returning then to its parent chromatid. The other "new" chromatid duplicates along the other parent and when it comes to the place that served as template for the first one, this has already separated off its piece so that nothing prevents the later arriving strand from duplicating along the same template.

This line of speculation then leads one to attempt to correlate the true crossovers with the abnormal segregation in a more direct manner than by saying merely that both are statistically favored by close pairing. On the contrary, one is led to the point of view that the irregular segregation displays the finer details of crossing over.

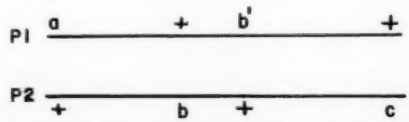

FIGURE 2.—A four factor cross involving two isolocal markers b and b′ and two outside markers a and c, as plotted in the modern picture.—Such a cross is for instance given in the paper of M. B. MITCHELL (1955a) where here stands

a for *pyr-1* b for *pdxp*
c for *co* b′ for *pdx*

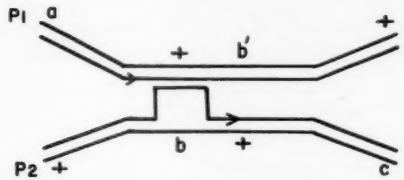

FIGURE 3.—A four factor cross resulting in a wild type segregant with respect to the isolocal markers for one strand and in three parental strands. Explanation by the switch hypothesis: One chromatid duplicates along the P2 parent, switches near the b^+ marker by chance over to the P1 parent duplicating along there for a while (or incorporating a preformed piece) and then switches back to the P2 parent. The other chromatid duplicating a little later along P1 finds the part around b^+ vacant again and thus proceeds duplicating along P1. No recombination is observable for the outside markers in this tetrad.

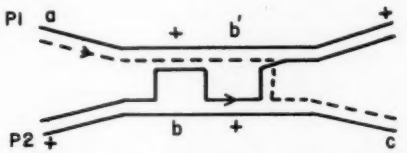

FIGURE 4.—Production of a three fold recombinant ++++ according to the switch theory. One chromatid replicates along the P2 parent, switches three times and then proceeds replicating along the P1 parent. The other chromatid, coming somewhat later along P1, finds the P1 parent vacant also in the region of the marker b^+, because the first chromatid has here already separated off. At a later point this chromatid finds the P1 parent occupied thus having only the P2 parent open for further replication. In this way the reciprocal appearance of the two recombinant types of more distant markers in a cross is obtained.

The general picture is this: In a region of close pairing (= crossover region) multiple switches take place, involving in any one region only two of the four strands (but the strands involved in different regions are not correlated). These switches may be and in general will be, nonreciprocal in detail, but they must be reciprocal at the ends of the region of intimate pairing, as illustrated in figure 4.

The switch hypothesis can explain the phenomena in small dimensions by just one mechanism, and it yields for larger marker distances the classical results of reciprocal crossovers: A region of intimate pairing containing an odd number of switches for each of two chromatids results in an observable "crossover", while an even number of switches in a chromatid is not detectable for classical marker distances. It is not yet clear whether a second mechanism, such as sister strand exchanges, is necessary for large dimensions to explain 4-strand crossing over, or whether the special nature of the duplicating molecule (DNA) makes it possible to understand by the same mechanism why randomly any two of the four strands are involved in any one region of intimate pairing.

It must be noted that the difference between the conversion hypothesis (once the notion of an order of isolocal markers within a gene has been accepted) and the switch hypothesis is only slight because in both hypotheses extra duplicated pieces are transferred. A decision could be made by a statistically significant number of

ascus dissections in a cross involving two isolocal and two close outside markers, because these theories differ in the *maximum* number of strands which can be involved in any one switch region. In the conversion hypothesis the conversion of a new strand should occur *during* the duplication and a "correlated" material exchange between any two of the four strands *after* the duplication. Therefore, *three* strands may be involved and those asci containing two strands recombinant for the outside markers should show a wild type segregant for the isolocal markers as often in a strand which is recombinant for the outside markers as in one which is parental. In contrast, the switch hypothesis assumes that only *two* strands are involved in recombination within any one region of intimate pairing. Therefore in those asci containing two strands recombinant for the outside markers the wild type segregant with respect to the isolocal markers should practically always be found in a strand having the recombinant type of outside markers. Some indication in this direction is given by the reported ascus dissections of M. B. MITCHELL (1956): in none of the four asci containing a wild type segregant for the isolocal markers are more than two strands involved in the production of "recombinants".

In all considerations the possibility that isolocal markers can be arranged linearly with respect to each other and with respect to outside markers plays an important role. This concept of order has not been verified experimentally except partially for phages (BENZER 1955; DOERMANN, personal communication) which may, but need not, involve the same recombination mechanism as other organisms. Therefore a study of three isolocal histidine mutants was undertaken, using crosses between pairs of these mutants in all three combinations and employing three outside markers. It was hoped to obtain in this way also some more information as to whether the correlation effect is produced by a mutation like phenomenon or by the insertion of larger chromosomal pieces.

The marker order (GILES 1955), the recombination frequencies of the distant markers and the kind of crosses performed are clear from the scheme given in figure 5. Not only two, but three outside markers were used because this allows one to distinguish histidine independent recombinants from several other possible types: wild type contaminations, pseudowild types (still carrying the *hist* markers) and heterocaryons (for instance, also between a histidine independent recombinant and a *hist* marker containing parent type). If such types would appear very frequently, one should very frequently obtain a mycelium that is neither dependent on inositol (*inos*) nor on p-aminobenzoic acid (*pab*), while for the real histidine independent recombinants less than 1 in 100 spores should be of this independence (since the map distance between *inos* and *pab* is one unit).

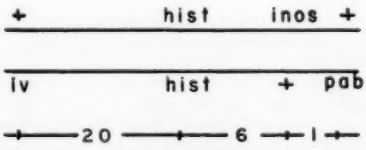

FIGURE 5.—Scheme of the crosses performed between pairs of *hist* markers and three outside markers. The three histidine markers are called *hist-1/1*, *hist-1/2*, *hist-1/3* or shortly 1, 2, 3. The properties and origins of all markers are given in table 1.

MATERIALS AND METHODS

The mutant strains were obtained from M. B. MITCHELL. The three histidine mutants (HAAS *et al.* 1952) should be isogenic, at least in the environment of the *hist* locus, because they were all produced from the same wild type. The origin and growth requirements of the different mutants can be seen from table 1. All mutants belong to linkage group V (BARRATT *et al.* 1954).

In order to obtain the different mating types or to eliminate an undesired growth behavior (e.g., microconidial fluffy) a mutant was crossed to wild p3178-2 a or wild p3177-4 A. The necessary double and triple mutants were obtained by crossing, plating the spores on very slightly supplemented medium, placing the smallest growing plants into growth tubes and later testing for biochemical deficiencies.

The heterocaryon tests between pairs of *hist* mutants were performed by putting very concentrated suspensions of conidia in water at one end of a "race" tube which was half filled with Fries minimal medium + agar (BEADLE and TATUM 1945) and containing either none or a limiting histidine supplement.

For crosses, supplemented WESTERGAARD-MITCHELL (1947) minimal medium was used in large tubes with filterpaper strips. Because all mutants containing the *iv* marker produced only a small number or no protoperithecia, always the *hist inos* double mutants were taken as protoperithecial parents in the final crosses. The cultures used for inoculation or fertilization were each time tested for their biochemical deficiency. For each cross another slant was taken and in this way a large number of independently prepared crosses obtained.

To select in the final crosses for the histidine independent recombinants, Fries medium supplemented with iv, inos and pab was used on plates. For plating the spores a soft agar layer technique was used in order to obtain an equal distribution of the spores over the plate and all spores in one plane, so that counting under the microscope was easy. The soft agar was the same supplemented Fries medium but with 0.5 percent agar. The spores were added to a bottle with liquid soft agar medium which was kept in a 45°C bath. The bottle was shaken strongly to separate all spores from each other and then onto each of as many plates as possible 2 ml of the liquid were pipetted and spread immediately by movements of the plate. The heavy spores

TABLE 1

The mutant strains and their properties

Marker	Isolation number	Growth on minimal medium	Mutagen used	Crosses grew best with the supplement, per 100 ml. of
hist-1/1	C91-R4	none	UV	20 mg histidine or more
hist-1/2	C84-R4	none	UV	same
hist-1/3	C85-R4	none	UV	10 mg histidine or more
inos-1	83201-s-5	grows in 25° none in 35°	X-ray	0.4 mg i-inositol
iv	39709	none	UV	30 mg L-isoleucine plus 40 mg L-valine
pab	1633	leaky	X-ray	4 γ p-aminobenzoic acid

The *iv* mutant grows slowly on supplemented medium; all other mutants grow like wild with the given amounts of supplement.

sink to the bottom of the soft agar layer before this can harden. Germination is induced by heating either the bottle before or the plates after the distribution of the soft agar for 40 minutes at 60°C. Spores containing the *hist* marker can just germinate in this medium, producing one or two very short hyphae. Only the histidine independent spores (and pseudowilds) can grow out further. At first we plated at most 10,000 spores per plate, scanned each plate after *ca* 20 hours growth at 25°C and picked the rare number of large growing plants which were all clearly originating from one spore. Biochemical tests showed that none of these plants was a wild or pseudowild type. Because neither with the heterocaryon test nor here had we observed heterocaryons, we had good reason to believe that the number of heterocaryons produced would at least be small when we altered the procedure by using somewhat more spores on a plate and the colony producing sorbose medium (supplemented Fries medium containing one percent sucrose and one percent sorbose) (see NEWMEYER 1954). In this sorbose medium the growth of the *hist* spores is as tiny as without sorbose while the larger growing plants produce colonies which can be seen with the bare eye after three to four days. Scanning some of these plates after two days in the microscope we observed that each little colony clearly arose from a single spore. Only then did we take more spores per plate. (The highest number was in one case 80,000!) This still gave usually none or one colony per plate, rarely more.

The hyphae of these colonies grow through the upper surface of the soft agar layer while the little hyphae of the hist deficient spores stay beneath. For the hist deficient spores no feeding by the colonies could be observed with the microscope. It can therefore be expected that the protruding hyphae of a colony are mostly free from hist deficient hyphae. The experimental results confirm this (see table 2). Thus these hyphae were picked and transferred into slants with Fries minimal medium supplemented with iv, inos and pab. After one or two days the plates were inspected once more. Occasionally one more colony was found on a formerly unopened plate. It was picked too and usually contained the *inos* or the *iv* marker.

Because of the large number of spores on the plates a special counting device was constructed which made it possible to count (with 36 fold magnification and a semi-dark field illumination) the number of germinated and ungerminated spores in four areas of given size, and to scan systematically the whole plate when this was necessary. Multiplying the counted number, which mostly was larger than 500, by a given factor gave a good estimate of the number of spores on one plate. The number of spores on all the other plates pipetted from the same bottle was then assumed to be equal. In a check the counting variation from plate to plate was less than 20 percent; but the concentration of the spores was slightly larger at the rim of the plate.

EXPERIMENTAL RESULTS

We first applied the heterocaryon test in order to see whether any of the three *hist* markers belong to different cistrons. In these tests no histidine independent heterocaryons have been found. This negative result shows that either no heterocaryons have been built at all or that the different histidine dependent nuclei within a heterocaryon cannot crossfeed each other.

For the final crosses the necessary double and triple mutants were produced and crossed in the described manner: *hist inos* × *iv hist pab*. In the crosses with the *iv hist-1/1* parent the *pab* marker was not present because lack of time had prevented its inclusion.

The spores of the final crosses were plated in the described manner on selective medium containing only iv, inos and pab supplements. Hyphae of the large growing plants were picked and tested for their biochemical deficiencies, and the numbers of germinated and not germinated spores were counted. The germination was generally larger than 70 percent.

The results of these crosses are summarized in table 2. The self-crosses show that spontaneous reversions of the *hist* markers are negligibly small. In all crosses with two different *hist* alleles histidine independent colonies occurred with a frequency of one to five in 100,000. The mycelia of the histidine independent colonies, when tested for the other biochemical deficiencies, proved to contain about equal frequencies of all four types of outside marker combinations (not considering now the *pab* marker). This shows that recombination between the outside markers is correlated with the appearance of *hist*$^+$ segregants, similar to the results already published by the aforementioned authors. In our case, similar to that of ST. LAWRENCE, but in contrast to those of MITCHELL and of GILES, the inequality between the two recombinant types of outside markers is very small, the difference in our numbers not being significant. This large correlation effect can certainly not be explained by the ordinary crossover theory, because from two factor crosses the expected probability of obtaining in

TABLE 2

Result of the crosses

Cross	Total living in 10^8	Hist. independent segregants	*iv inos* recombinant	++ recombinant	*inos* parent	*iv* parent	Wild type (contaminants?)
1 × 1	0.66	0	—	—	—	—	1
2 × 2	1.41	0	—	—	—	—	0
3 × 3	2.80	0	—	—	—	—	0
1 × 2	4.82	35	7	5	11	10	2
2 × 1	3.21	49	8	13	11	17	some of the plants counted as ++?
1 × 3	2.62	73	15	11	24	22	1
3 × 1	2.18	62	10	14	21	17	some of the plants counted as ++?
2 × 3	1.83	101	30	25	24	20	2
3 × 2	2.66	120	20	27	44	29	1

The first parent in the cross is always the *hist inos* parent, the second the *iv hist pab* parent. In the cross *hist-1/2 inos* × *iv hist-1/1* = 2 × 1 and the cross 3 × 1 the *pab* marker was not present. In the crosses containing the *pab* marker no type containing either the *pab* or the *inos* marker was found besides those counted as contaminants. Only in the cross *hist-1/3 inos* × *iv hist-1/2 pab* = 3 × 2 two types have been found containing both the *inos* and the *pab* marker. One of them (*inos pab*) was counted as *inos* parent, the other (*iv inos pab*) as *iv inos* recombinant. In the crosses containing the *pab* marker all the "++ recombinants" and the "*iv* parents" carry also the *pab* marker which for convenience was not written in the table.

addition to a crossover between two *hist* markers a crossover in the *iv. . .hist* region would be 0.2 and in the *hist. . .inos* region 0.06. Thus a triple recombinant should occur only in about 0.012 of all cases.

The biochemical tests showed that for crosses involving the *pab* marker none of the colonies contained hyphae which, carrying the *iv* marker, were independent of both inositol and p-aminobenzoic acid. Therefore the number of colonies containing a heterocaryon between histidine independent nuclei and nuclei containing one of the parent type of marker combinations seems in our case negligibly small.

The crosses containing the *pab* marker gave in a total of 329 histidine independent colonies altogether six colonies whose conidia behaved biochemically as wild type (two of them growing slightly better with than without inositol). Thus the number of wild type contaminants or heterocaryons was small and it can be expected that this number was also small in crosses with the *iv hist-1/1* parent (without *pab*). These six types were backcrossed to wild, the spores plated on minimal medium and incubated for 12 hours at 35°C. For four types all observed germinating spores gave wild type growth, which was also assured by testing some of them biochemically. The two types growing slightly better on inositol came from crosses between *hist-1/2* and *hist-1/3*. They evidently contained mostly inositol nuclei (besides wild types) because the ratio of inositol dependent and independent spores was 5:3 in one and 5:2 in the other case. It might be that originally *inos* colonies were contaminated with wild type after picking. In any case neither in the heterocaryon test nor in the crosses have wild type heterocaryons (or pseudowilds) between any two different *hist* markers been detected. Thus the three *hist* markers belong to the same cistron.

DISCUSSION

Since in crosses with different *hist* markers the frequency of histidine independent segregants is rather different, one is tempted to arrange linearly the isolocal markers with respect to each other (fig. 6). A monotonical increase of recombination frequency with marker distance, allowing an ordering of the markers, is expected from the modern version of the conversion model as well as from the switch model. In the classical picture, however, which assumes that chromatid exchanges can occur only between the genes, an order of three isolocal markers could be obtained only if each mutation belonged to a different gene. This seems improbable in our case because no heterocaryons have been found between any two *hist* markers and there is no biochemical evidence that this *hist* locus should consist of three biochemically functional units (genes). All three mutants are blocked in the production of the same enzyme. See Ames (1957).

In classical genetics it is postulated that the recombination frequencies are additive for marker distances that are not too long. This certainly cannot be expected to be true for markers whose distances are comparable to the average length of a region of intimate pairing since a wild type segregant can appear not only when this region lies between the markers, but also when it is overlapping one of them. Thus the recombination frequency is always expected to be a little larger than the "map distance" or, in other words, the sum of the two smaller recombination frequencies between three markers is expected to be larger than the largest recombination fre-

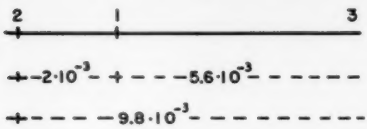

FIGURE 6.—Recombination frequencies between pairs of *hist* markers in percent. The values in reciprocal crosses differ only slightly in crosses 1 × 3 and 3 × 1 or in 2 × 3 and 3 × 2, while in the cross 2 × 1 the recombination frequency is twice the one of cross 1 × 2. These variations may be considered as a measure for the heterogeneity of the genetic background.

quency (except when one of the mutations involves an appreciable deletion). For our crosses the experimental error is too large to allow a significant statement about additivity.

The proposed possibility of arranging our three *hist* markers in a linear order can only be regarded as a further indication against the classical view but not as a proof. For it is well known from classical genetics that variations in the genetic background may give rise to recombination frequencies from which a wrong marker sequence is deduced. Because the used double and triple mutants are fairly isogenic such a wrong inference seems not probable in our case but an independent proof would be convincing. The ordinary kind of accurate test, three factor crosses, seems at present not feasible for Neurospora, using the isolocal markers alone, because it is very difficult to isolate a double mutant without a selective technique. However, it should be possible to determine the order of the isolocal markers from crosses involving one or two outside markers, although the correlation effect blots out somewhat the frequency differences. When only one outside marker is used, the correlation effect is mostly so pronounced that it is hard to obtain a statistically significant difference between the two types which are wild type segregants for the isolocal markers. Thus the ordinary three factor test should for isolocal markers be replaced by a four factor test involving two outside markers. This can be seen in the cases studied by MITCHELL (1955 a,b) and by GILES (1955) where the two recombinant types of outside markers occur with very different frequencies. For these cases it seems reasonable, considering that the mentioned possible models explain the correlation effect, to propose the following rule for determining the order of two isolocal markers with respect to the outside markers: The more complicated events within a region of intimate pairing give rise to the less frequent recombinant type of outside markers. This definition leads to the same marker sequence whichever of the proposed models is used. For MITCHELL's (1955a) case one obtains, for instance, the order *pyr-1 pdxp pdx co*. The same order should result from the "reciprocal" cross (in which just the two isolocal markers are interchanged). Indeed, this is the case in the crosses of MITCHELL and of GILES. The proof for the proposed rule should either come from its consistency in several crosses with different isolocal markers or by comparison with a three factor cross involving three isolocal markers.

In our experimental results, as in the crosses reported by ST. LAWRENCE (1956), the frequency difference of the recombinant types of outside markers is small, probably because the isolocal markers are very close to each other. Therefore, the difference in our numbers is not significant; but these numbers are in reciprocal crosses at

least compatible with the assumption that the markers 1 and 2 are closer to the *iv* locus than is marker 3. A convincing proof for the modern view point has in the meantime been given by the author, using the experimental results with a *pab* locus. (Freese 1957.)

In the introduction we raised the question of whether the frequent appearance of the parental type of outside markers in combination with wild type for the isolocal markers is produced by a "directed backmutation" or by an insertion of a larger chromosomal piece into the homologous chromatid (as in the transfer or the switch model). From our experiments the directed backmutation seems improbable: In this model the probability for the appearance of a directed backmutation should be a specific property of the mutated place alone (because our strains are isogenic at least in the neighborhood of this chromosomal part) and should at least not depend in a regular fashion on the distance of other markers. The frequency for the occurrence of a parent type of outside markers should then be approximately equal to the probability f_i that the marker *hist-1/i* has been back mutated to wild. Grouping together always reciprocal crosses with their proper statistical weight, we get for the different crosses values for the f_i which can be seen in table 3. There are striking differences in the estimate of the same f_i in two different crosses (especially for f_2). It therefore seems improbable that directed backmutations are the cause for the frequent appearance of parental types of outside markers, unless the intimate pairing is appreciably decreased in the neighborhood of a mutation. Such a decrease of pairing seems improbable as long as a mutation involves only a change of a small number of DNA nucleotides. In our case for instance the correlation effect extends over the whole distance between our most distant *hist* markers and therefore the pairing area must be at least as large as this distance. This involves a large number of DNA nucleotides and thus pairing should not be influenced appreciably by the change of just some of them. Therefore it seems very probable that larger chromosomal pieces, duplicated along one parent, can be inserted into the new chromatid duplicating along the other parent. This qualitative argument is not affected by the possibility of independent crossovers outside the *hist* region (the correction being small).

TABLE 3

Values of f_i in units 10^{-6}

Cross between:	1 and 2	1 and 3	2 and 3
f_1	3.5	8.7	—
f_2	2.6	—	11.8
f_3	—	9.1	14.3

f_i is the frequency of those histidine independent segregants which have the same combination of outside markers as the parent containing the *hist-1/i* (marker).

e.g. In the cross *hist-1/1 inos* × *iv hist-1/2 pab* f_1 is the probability to obtain *inos* and f_2 the probability to obtain *iv pab* among the total number of germinating spores. From table 2 one obtains the following weighted average of f_1 from the crosses 1 × 2 and 2 × 1:

$$f_1 = \frac{11 + 17}{4.8 + 3.2} \cdot 10^{-6} = 3.5 \cdot 10^{-6}$$

The foregoing considerations make it probable that either the transfer model (accepting the possibility of an order of isolocal markers) or the switch model or something similar are the right explanations for the observed phenomena in small dimensions.

If the statement that chromosomal pieces are sometimes duplicated along one strand twice while not at all on the homologous strand should prove correct, a severe limitation would be placed on models trying to explain the duplication of chromosomes.

SUMMARY

A genetic study involving three closely linked histidine requiring mutants and three other linked markers has been undertaken. Histidine independent heterocaryons (or pseudowilds) between any two histidine mutants have not been found. Crosses between any two different histidine mutants yield rare histidine independent progeny while self-crosses do not. The recombination frequencies suggest an order for the three histidine markers with respect to each other. The histidine independent progeny contains parental and both recombinant types of outside markers with about equal frequency. These results cannot be explained by classical genetics. A reason has been given why the events occurring in small regions of intimate pairing and resulting in the observed correlation effect seem not to involve mutation-like phenomena but rather the insertion of chromosomal pieces, replicated along one parent, into the homologous chromatid. How the incorporation of this piece comes about is a question of more specific models, some of which have been mentioned in the introduction. A model-independent rule for ordering pairs of isolocal markers with respect to the outside markers has been given which is similar to the rule of classical genetics.

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PHENOTYPIC VARIABILITY AND THE INFLUENCE OF TEMPERATURE ON SOMATIC INSTABILITY IN CULTURES DERIVED FROM HYBRIDS BETWEEN NICOTIANA LANGSDORFFII AND N. SANDERAE[1]

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PLANT cultures showing variegation for flower color have originated, as described by SMITH and SAND (1957), following the interspecific hybridization of *Nicotiana langsdorffii* and *N. sanderae*. Genetic studies conducted with one of these derived cultures, variegated-1, have shown that the variegated phenotypes and *N. sanderae*, which is nonvariegated, differ from each other in the alleles present at a single, major locus. Solid-colored *N. sanderae* has the constitution V/V. The principal variegated-1 phenotypes are speckled, v_s/v_s; sectorial, v_S/v_s; and rare sectorial, v_S/v_S. The genetic data are interpreted to indicate that v_s and v_S are unstable alleles which may undergo reversible changes between their alternative forms in the lineage of certain germ cells thus modifying the genetic ratios among progeny. Also, these events occurring in the cells of the developing petal epidermis give rise to color variegation of the mature flowers. Differences in frequency and developmental timing of these events lead to different phenotypes of variegated flowers. This variability has both heritable and environmental components. The studies reported here were designed to estimate the magnitude of several causes of this phenotypic variability and to investigate the effect of temperature during development as a contributing factor.

MATERIALS

In these studies three major phenotypes of variegated plants were employed: the speckled and sectorial phenotypes of a variegated-1 culture and the flecked phenotype of a variegated-3 culture. Both cultures have been derived by selection and backcrossing to *Nicotiana sanderae* following interspecific hybridization with *N. langsdorffii*.

The variegation phenomenon concerns the distribution of anthocyanin pigmentation in the epidermal cells of the petals. The corolla of nonvariegated *N. sanderae* is uniformly solid red in color. The speckled phenotype has scattered, small clusters of red cells on a background of colorless cells. The sectorial phenotype has frequent, clearly defined sectors of speckled tissue on a background of solid red tissue. This sectoring of variegated-1 may occur over a wide range of time during plant or flower development. Thus a speckled sector may involve only a few petal cells or it may include a large portion of the petal epidermis of a flower, branch or plant. The variegated-3 (flecked) phenotype resembles the variegated-1 (sectorial) phenotype in

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possessing sectors of speckled tissue on a background of solid red tissue. However, variegated-3 shows a dramatic difference in the timing of the sectoring events. In this line the sectors are frequent but medium to small in size, giving a flecked appearance to the petals. Thus large sectors are extremely rare in the flecked phenotype, presumably because sectoring events occurring early in development are rare in this line.

OBSERVATIONS

Phenotypic variability

Variation in speckling-intensity phenotype among sister plants. It has been found possible to score flowers for speckling intensity by estimating the frequency of pigmented epidermal cells. A corolla lobe of a fresh flower is viewed at high-dry power through a microscope employing a green filter. It is possible to count the number of pigmented cells per microscope field of view. With the microscope and magnification used, one field of view encompassed approximately 200 epidermal cells.

During a period of six days in the summer of 1952 20 speckled plants (progeny from self pollinating one speckled plant) were scored in the above manner. These results are reported by SMITH and SAND (1957).

The data are not considered exhaustive enough to permit detailed analysis of differences among individual plants. It is indicated, however, that the variegated-1 culture contains speckled plants whose flowers vary in their average frequency of pigmented epidermal cells. Other studies have shown that at least a portion of this variability has a genetic basis.

Variation in speckling-intensity phenotype with temperature during development. A clone of speckled plants was established in the fall of 1952. When beginning to flower two members of the clone were placed in each of three different constant temperature greenhouses at 55°F, 70°F, and 80°F. Scoring was begun four days after initiating the constant temperature treatments. During the ensuing 12 days three flowers were scored from each of the six plants, employing the technique described above for estimating speckling intensity.

Analysis of variance has demonstrated highly significant differences in speckling intensity among the three temperature treatment means. The linear regression on temperature is highly significant and deviations from regression are not significant. It is concluded that the speckling intensity of flowers of the tested clone is temperature sensitive. The response is linear within the temperature range employed. An increase in temperature is accompanied by a decrease in the frequency of pigmented cells in the petal epidermis (fig. 2). Other speckled plants of the variegated-1 culture have not been tested under controlled conditions, but field observations give the impression that the temperature response is a general phenomenon.

Variation in speckling-intensity phenotype with age of flower. Members of two clones of speckled plants were used in the spring of 1954 to provide material for an estimate of the change in speckling intensity that occurs as a mature flower grows older on the branch.

Five plants were employed from each clone and two branches from each plant. From each selected branch the three most terminal blooms were harvested on one day. These blooms were designated as flowers 1, 2, and 3 in order from the branch

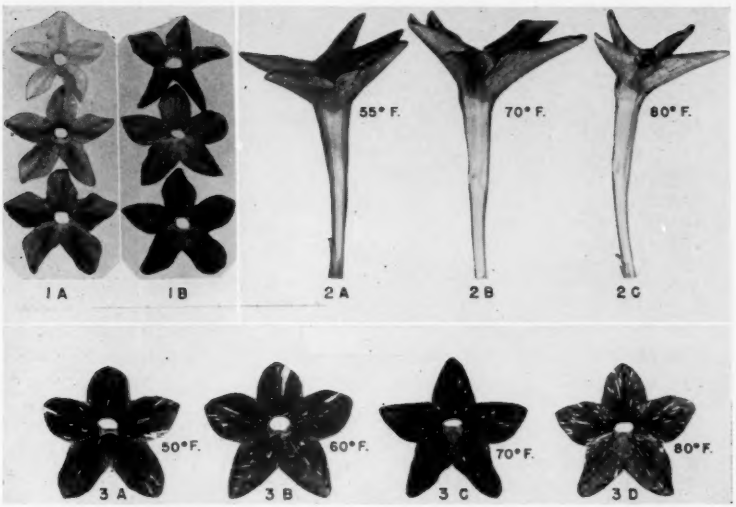

FIGURE 1.—Flowers of different ages from two clones of variegated Nicotiana. Speckling intensity increases from top to bottom with increasing age of flower.

FIGURE 2.—Flowers from a clone of variegated Nicotiana cultured at different controlled temperatures. Speckling intensity decreases with increasing temperature.

FIGURE 3.—Flowers from clone S5230-5 of variegated Nicotiana cultured at different controlled temperatures. Sectoring frequency is highest at 80°F.

apex. The most apical flower is the youngest. Thus ten flowers of each of three age-classes were harvested from each clone.

The Hunter Color Difference Meter calibrated to a white standard was used to measure the total reflectance of a 1.5 square centimeter area on each corolla. Total reflectance measurements were taken also on flowers of solid red and solid white phenotypes.

For each clone the mean total reflectance values were calculated for each flower age. Total reflectance was interpreted as an inverse measure of speckling intensity: the greater the reflectance the lighter the speckling.

The results show that speckling intensity increases with increasing flower age within each clone (fig. 1). The oldest blooms of the light-speckled clone are darker on the average than the youngest blooms of the dark-speckled clone. Thus a valid comparison of speckling intensity between plants requires the use of flowers of the same age.

Variation in sectoring-frequency phenotype among plants. In the summer of 1952, 127 flowers from nine sectorial plants of the variegated-1 culture were scored for their sectoring frequency. For this purpose the sectors were divided into two size categories: (1) small sectors, the points of origin of which can be seen in the petal lobe, and (2) large sectors, which originate at points that are not visible in the petal lobe, i.e., in regions of earlier development of the flower or branch.

Both small and large sectors were counted on the adaxial surface of each lobe of

TABLE 1

Sectoring-frequency variation among sectorial plants of variegated-1

Plant pedigree number	(n) Number flowers scored	(A) Mean large sector degrees per flower	(B) Mean small sector number per flower	(C) Adjusted† mean small sector number per flower	($s_{\bar{x}}$)‡ Standard error of adjusted mean
*S527-3	3	3.7	329	332	23.7
*S527-15	12	84.7	221	288	11.8
*S527-25	15	3.2	281	283	10.6
S526-11	11	79.1	216	277	12.4
*S527-36	30	54.8	226	266	7.5
S529-1	9	12.9	236	245	13.7
*S527-27	30	77.3	191	243	7.5
*S527-22	8	23.9	212	227	14.5
*S527-34	9	2.3	197	198	13.7
Total	127	49.1	223	258	3.7

* = Seven full-sister, sectorial plants.

† = Calculated using: $C = 360\ B/(360\text{-}A)$.

‡ = Error variance for adjusted sector number = $s^2 = 1{,}684$ (117 degrees of freedom).

$s_{\bar{x}} = \sqrt{s^2/n}$ used in calculation.

every flower. When the lobes of a flower are seen at full face view, the large sectors take the form of wedges of tissue emanating from the center of the flower. Thus such large sectors may vary in angular size from a few degrees to 360 degrees when they include the whole flower. For each large sector the angular width in degrees and the position of its limiting radii on a 360 degree reference circle were recorded.

When large sectors occur, the count of small sectors will be reduced in proportion to the area in large sectors. Thus in order to compare the small sector frequencies of the different plants their small sector averages have been adjusted to the values that would have been expected had no large sectors been present. A summary of this portion of the data is presented in table 1.

It is considered that the unequal and relatively small numbers of flowers scored from different plants prevent appraisal of precise differences in sectoring frequency between specific plants. Nevertheless, the variability is large among the adjusted plant averages. These data indicate that certain sectorial plants of the variegated-1 culture differ from each other in their frequencies of small sectors.

Other studies support this conclusion of heterogeneity of the population of sectorial plants. In results reported by SMITH and SAND (1957) genetic tests of two sister sectorial plants ($v_s v_s$) have shown correlation between the frequency of sectors of speckled somatic tissue ($v_s\ v_s$) and the frequency of speckled progeny ($v_s\ v_s$) obtained from self pollinating the respective plants.

Large-sector size distribution. As discussed in the previous section, large sectors have been defined as those not entirely included within the area of a mature petal lobe. Since these large sectors take the form of wedges of tissue, the angular width in degrees was used as a measure of the size of each large sector.

Of 131 flowers scored, 51 possessed at least one large sector. The size of these sec-

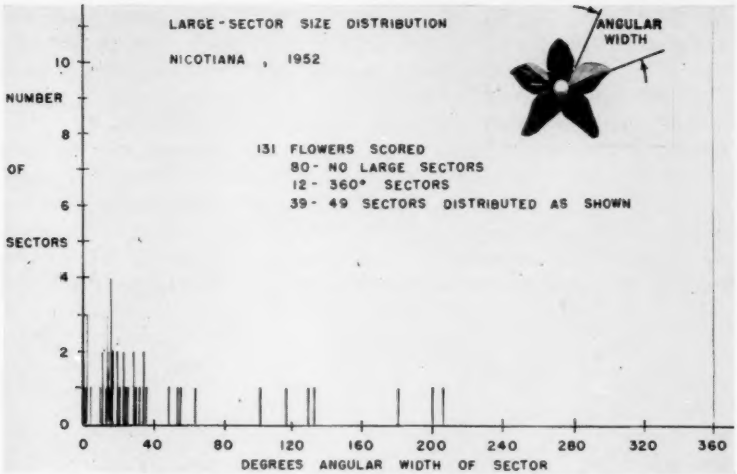

FIGURE 4.—Size (angular width) distribution of large sectors on petals of variegated Nicotiana. The distribution is discontinuous.

tors ranged from a few degrees to 360 degrees; more than half of the 61 sectors were less than 40 degrees in angular width. While the data are a minimum, a pattern of discontinuous distribution emerges (fig. 4). There exist deficiencies in the distribution representing sector sizes which are not as frequently produced as others. These deficiencies are clear in the regions centered at 80 and 160 degrees, and extend over a range of about 40 degrees in each case. In addition no sectors between 210 and 360 degrees in size were found on the flowers scored. Although it is known that sectors in this large size range do occur, they are relatively infrequent. In conjunction with the gaps in the distribution there are clusters with sector modes near the sizes 60, 120, and 190 degrees.

It is proposed that such a distribution lends support to a concept that the sectors delineate ontogenetic cell lineages. These data suggest that at a very early stage in floral development there exists a definite number of primordial cells whose descendants will constitute all of the epidermis of the mature corolla. These cells would have to be sought, near the time of initiation of the petal primordia, at a stage in development earlier than was studied by AVERY (1933) for the tobacco leaf.

For example, we may consider the existence of six initials, (or of six primordial cells derived from a single initial) each destined under normal conditions to produce one sixth of the mature corolla epidermis. Then a sectoring event occurring in one of these cells should result at floral maturity in a sector occupying one sixth of the corolla or 60 degrees of petal epidermis. If two adjacent initials were of the altered type (probably due to a sectoring event in a common ancestral cell) then a 120 degree sector should result. Under such circumstances it would be expected that sectors between 60 and 120 degrees would be observed at frequencies much less than those

for the modal sizes. The data approximate this condition. Thus the discontinuous distribution of sector size favors the hypothesis of a definite number of corolla epidermis initials in these plants. It is also indicated that these initials may be no more than six in number, and that an orderly pattern of cell lineage connects these initials with their mitotic descendants in the epidermis of the mature corolla.

Large-sector position distribution. The position of a large sector on the mature corolla can be defined in terms of the locations of its limiting radii or marginal limits. The locations of 94 such marginal limits were recorded by noting whether they occurred (1) in one of the five petal lobes but not along a mid-lobe line, (2) along one of the five sinus lines between lobes, or (3) along one of the five mid-lobe lines. This position distribution of sector limits is summarized below:

	Lobe 1	Lobe 2	Lobe 3	Lobe 4	Lobe 5	Sinus line	Mid-lobe line
Origin	6	6	8	7	9	9	2
Termination	5	8	9	7	7	9	2

The data indicate that each of the five lobes of the corolla is equally likely to contain the margin of a large sector. However, a large sector is much more likely to have a limit along one of the five sinus lines between lobes than along one of the five mid-lobe lines. In fact, despite the much smaller area involved, as many sectors have origin or termination radii in a sinus as within any one entire lobe.

It is not thought that this observation indicates any different predisposition toward sectoring of sinus versus lobe lineage cells. Rather, it is considered to reflect a different pattern and frequency of cell division in the two lineages, which function to generate the five-lobed form of the mature corolla. Apparently there are fewer late, intussusceptive divisions of cells near the sinus lines than there are near the mid-lobe lines. If such divisions were to occur near the sinus lines they would be expected to alter the five-lobed morphology of the corolla, and to disperse the sector limits from their observed concentration along these sinus lines. The tendency for sector limits to coincide with petal lobe limits may indicate relatively independent cell lineages for the five petals soon after corolla initiation. This observation can be interpreted as evidence that these sympetalous flowers are morphogenetically polypetalous.

Temperature effect on sectoring

Materials and methods

As described above, two cultures of sectorially variegated *Nicotiana* plants are available, each of which contains individuals whose petals possess sectors of speckled epidermal tissue on a background of solid red tissue. Sectorial plants of the variegated-1 culture show the presence of some very large sectors as well as small sectors. On the other hand the sectors are much more limited in size range in the variegated-3 culture. Most of the latter sectors are small enough to be entirely included within the petal lobe in which they occur. This fact permits the petal lobe to be the sampling unit for measuring the frequency of sectors in the case of variegated-3 plants. Be-

cause of this convenience a clone of variegated-3 was used in the following study, summarized previously (SAND 1956).

Cuttings were taken in October, 1953 from a member of the clone S5230-5. These cuttings were rooted, potted individually, and kept together in the greenhouse until the cuttings began to flower. On January 1, 1954 two members of the clone, selected at random, were placed under each of four different constant temperature greenhouse conditions: 50°F, 60°F, 70°F, and 80°F. Two additional cuttings were continued under the previous greenhouse conditions in which the temperature was not automatically regulated. Flowers were harvested from these ten plants as they bloomed, and data were taken from each flower. Harvesting was continued until at least 21 flowers had been obtained from each plant. Other flowers were self-pollinated on each plant.

Each of three petal lobes per flower were scored for: (1) lobe width, (2) number of proximal points of speckled sectors in one square centimeter, and (3) number of epidermal cells per 100 ocular micrometer divisions.

The technique employed was to dissect the petal lobe and place it on a 4″ x 5″ glass plate. It was carefully covered with a microscope slide and briefly pressed by a lead weight. After removing the weight, the lobe width at its maximum point was measured to the nearest tenth of a centimeter. This temporary mount was then viewed by reflected light at 30× under a binocular dissecting microscope. A square, approximately one centimeter on a side, etched on the microscope slide, framed a corresponding area on the petal beneath it. This permitted the number of speckled sectors in 1.09 square centimeters near the center of the lobe to be counted. Only sectors containing eight or more cells were counted. The petal was then remounted on a glass slide under a cover glass. This preparation was viewed by transmitted light at high dry power under a microscope supplied with an ocular micrometer. The micrometer was rotated until its line was parallel to the long axis of the epidermal cells in view, whereupon the number of cells included in 50 divisions was counted. The micrometer then was rotated 90 degrees and the count was repeated. Record was kept for each lobe of its position on the flower, and for each flower of its date of harvest and of the plant and branch from which it was removed.

Results of temperature effect studies

General results. Several facts of general interest became obvious during the course of the experiment. Primary among these were the following: (1.) The variation of speckled-sector frequency within temperature treatments was large and showed trends rather than random variation during the course of the experiment. (2.) A larger flower size resulted at the lowest temperature. (3.) A near absence of pollen existed after about forty days of treatment at 50°F. (4.) A markedly different rate of bloom was correlated with temperature differences. At 80°F a branch would produce a mature flower on an average of every 1.4 days, whereas at 50°F an average of 3.2 days elapsed between blooms on a branch.

The experiment was discontinued upon obtaining 21 flowers from each of the 50°F plants. The data analyzed are for the first 21 flowers scored from each plant, although additional flowers were measured from the more rapidly blooming treatments. The

TABLE 2

Temperature treatment means calculated from data for three petal lobes from each of 42 flowers per treatment

Temperature treatments F°	Speckled sectors per cm^2	Epidermal cells per 10^{-4} cm^2	(Petal width)^2 cm^2	Speckled* sectors per 10^4 epidermal cells
50	8.0	7.52	3.1	0.98
60	10.2	8.33	2.9	1.18
70	10.1	9.45	2.6	1.11
80	11.4	9.90	2.7	1.23
Average	9.91	8.802	2.81	1.126†

* = Adjusted to the average number of epidermal cells per unit petal area by using the average error regression line.

† = A minimum estimate of somatic mutation rate per 10^4 cells is obtained by multiplying the tabulated value by 8.

TABLE 3

Summary of mean squares and significance levels obtained from analyses of variance of data for three variates from controlled temperature experiment

Source	d.f.	Speckled sector frequency	Epidermal cell population density	Petal lobe width
Temperature treatments	4	774.2†	1,932.0†	0.47†
Manual *vs.* automatic	1	2,197.1†	41.9 n.s.	0.01 n.s.
Among 4 automatic	3	299.9†	2,562.0†	0.62†
Linear regression on temp.	1	769.5†	7,498.6†	1.48†
Deviations from regression	2	65.1 n.s.	93.7*	0.20†
Petal lobes	2	326.0†	10.9 n.s.	0.32†
Lobe 1 *vs.* lobes 2 + 5	1	651.5†	—	0.64†
Lobe 2 *vs.* lobe 5	1	0.5 n.s.	—	0.00 n.s.
Harvest orders	20	32.8 n.s.	119.9†	0.022†
Treatments × orders	80	104.4†	78.0†	0.026†
Remainder	523	—	25.6	0.0087
Treatments × lobes	8	27.6 n.s.	—	—
Lobes × orders	40	12.7 n.s.	—	—
Treatments × lobes × orders	160	10.1 n.s.	—	—
Residual	315	40.1	—	—

* = Significant at five percent level.

† = Significant at one percent level.

n.s. = Not significant at five percent level.

21 orders represent order of harvest and have the significance of flower blooming sequence or relative node position. Each datum is classified therefore for lobe position, order of bloom, plant number, and temperature treatment.

Figure 3 shows flowers of variegated-3 harvested from the four constant temperature treatments 52 days after beginning treatment of the clonal cuttings. The high frequency of sectoring in the flower from the 80°F treatment is evident. Figure 5 shows a dissected petal lobe of a variegated-3 flower.

TABLE 4

Summary of mean squares and significance levels obtained from two analyses of covariance of data from controlled temperature experiment

Source	d.f.	Speckled sector freq. adj. for covariance with cell population density	Lobe width adjusted for covariance with cell population density
Temperature treatments	4	—	0.1600†
Among 4 automatic	3	115.2†	—
Linear regression on temp.	1	186.9*	—
Deviations from regression	2	73.1 n.s.	—
Petal lobes	2	323.1†	0.3150†
Harvest orders	20	34.6 n.s.	0.0120 n.s.
Remainder + treatments × orders	602	—	0.0100
Treatments × orders	80	103.0†	—
Remainder	522	28.3	—
Regression treatment means *vs.* average error regression	1	161.4*	0.45†

* = Significant at five percent level.
† = Significant at 1 percent level.
n.s. = Not significant at 5 percent level.

The data are summarized in tables 2, 3 and 4, and in figures 6, 7, 8, 9 and 10. These data indicate an effect of temperature upon the frequency of sectoring. This sectoring variable is of primary interest, however its interpretation depends upon simultaneous consideration of variation in epidermal cell size and petal size. These analyses are presented in the following sections and their interrelationships are considered further in the later interpretation.

Temperature treatment effects. Figure 6 shows the regressions on temperature of the three variates: (1) speckled sectors per unit petal area, (2) epidermal cells per unit petal area, and (3) petal area. In addition is shown the regression of speckled sector frequency on temperature after adjusting to the mean population density of epidermal cells. The ordinate values are represented as a percent of the experiment mean for each variate in order to facilitate comparison among the trends. Sectoring frequency and cell population density increase with temperature, whereas petal size decreases.

The temperature treatment means are presented in table 2. The values for sector frequency are shown in units of sectors per square centimeter. Cell size or number of cells per unit length was used to give an estimate of epidermal cell population density expressed as cells per unit petal area. This was accomplished by multiplying together the number of cells per 50 ocular micrometer divisions measured along a line *parallel* to the cells' long axes (cell lengths per 50 ocular micrometer divisions) and the number of cells per 50 ocular micrometer divisions measured along a line *perpendicular* to the cells' long axes (cell-widths per 50 ocular micrometer divisions). This product estimates the number of cells per 2500 square ocular micrometer divisions, or cells per 0.042 square millimeters. The experiment estimates an average of 8.8×10^4 epidermal cells per square centimeter. Measurements for the third variate, lobe width, were squared to give items which should be a function of lobe area. The

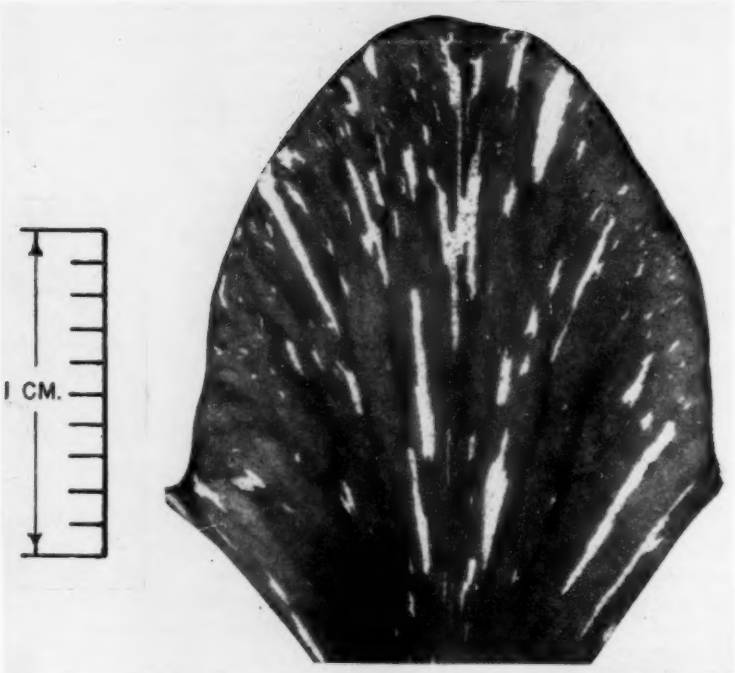

FIGURE 5.—Dissected petal lobe of a flower from clone S5230-5 of variegated Nicotiana. The smaller sectors are more frequent near the petal margins.

last column of table 2 contains values for speckled sector frequency adjusted for variation in epidermal cell size.

Table 3 summarizes the results of analyses of variance. Speckled sector frequency shows a highly significant linear regression on temperature with nonsignificant deviations from regression. Strong linear trends correlated with temperature treatments also are evident for epidermal cell population density and petal lobe width. These trends are generally descriptive of the temperature effects despite significant departures from linearity for the latter two variates.

The variation in epidermal cell size may be conceived as capable of affecting both petal lobe width and the frequency of sectors scored per unit petal area. Therefore, the analyses of covariance summarized in table 4 were performed. There exists a positive correlation between the linear regression of speckled sector frequency on temperature and the linear regression of cell population density on temperature. Nevertheless, after adjustment of the former for covariance with cell population density, there still remains a statistically significant linear regression of sector frequency on temperature. Adjusted deviations from this linear regression are not significant. Table 4 also demonstrates that the temperature treatment means' regres-

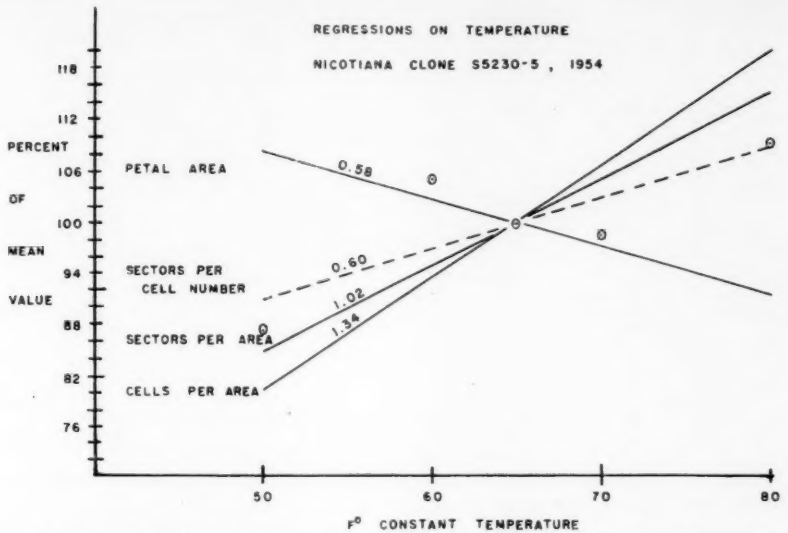

FIGURE 6.—Regressions on temperature of the three variates measured. The dotted line and the points shown are for the regression of speckled sector frequency on temperature after correcting for variation of cell number in the petal areas sampled.

sion of sector frequency on cell population density is significantly greater than the corresponding average error regression. This relationship is shown in figure 7. Apparently temperature has an effect on sectoring frequency greater than the net effect due to the uncontrolled factors in the experiment which influence cell population density. Similar considerations from table 4 indicate that the temperature effect on lobe width cannot be entirely accounted for by correlated changes in cell population density.

Interaction effects of temperature treatments and orders of harvest. The different orders or periods of harvest do not show significant variation in sector frequency when averaged for all the temperature treatments. This is shown in table 3 which indicates, however, that the corresponding order effects for cell size and lobe width are statistically significant. These latter effects are correlated with each other: Table 4 shows that the harvest order means for lobe width do not differ significantly after being adjusted for covariance with cell population density.

Table 3 shows that sector frequency, cell population density, and lobe width all give a significant interaction of temperature treatments and orders of harvest. These interactions indicate that differences among the temperature treatment responses change during successive periods of harvest. The most pertinent of these interactions, that for speckled sector frequency, is shown in table 4 to remain statistically significant after adjustment for covariance with cell population density.

Figure 8 graphically represents the increase in the regression of speckled sector frequency on temperature during progress of the experiment. For purposes of clarity

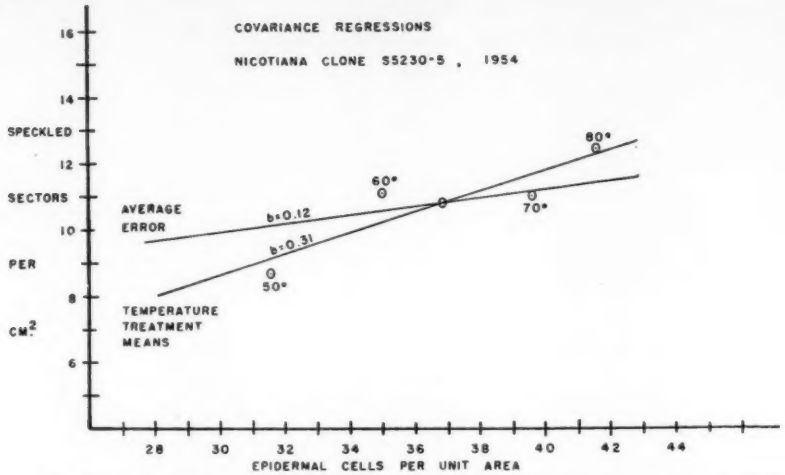

FIGURE 7.—Covariance regressions. The regression of temperature treatment means is significantly greater than the average error regression.

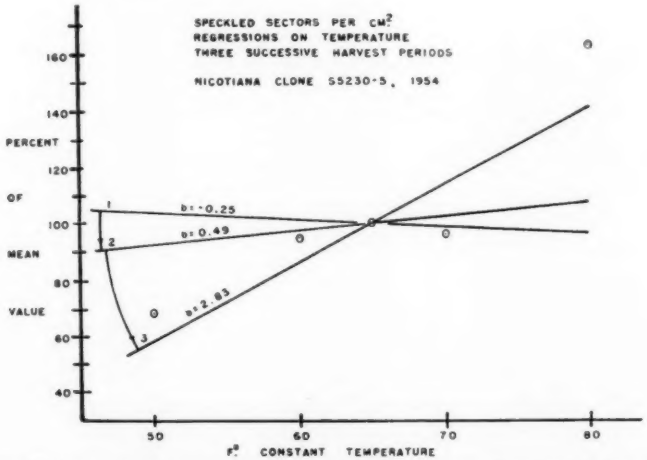

FIGURE 8.—Increase in the regression of speckled sector frequency on temperature during progress of the experiment. The points shown are for the third harvest period.

the 21 orders were divided into three successive harvest periods of seven orders each, and regressions were calculated for each of the three order series. The correlation between higher sectoring frequency and higher temperatures increases in successively later times of harvest.

Figure 9 by comparison shows that there is no distinguishable alteration in the

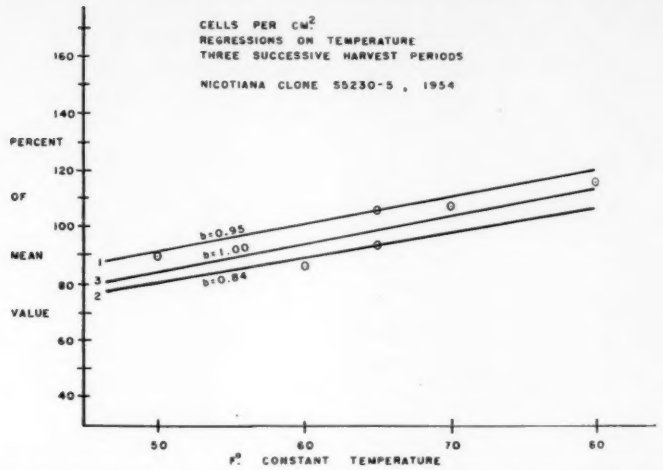

FIGURE 9.—Constancy of the regression of cell population density on temperature during progress of the experiment. The points shown are for the third harvest period. Figures 8 and 9 together indicate an effect of temperature on sector frequency independent of its effect on cell size.

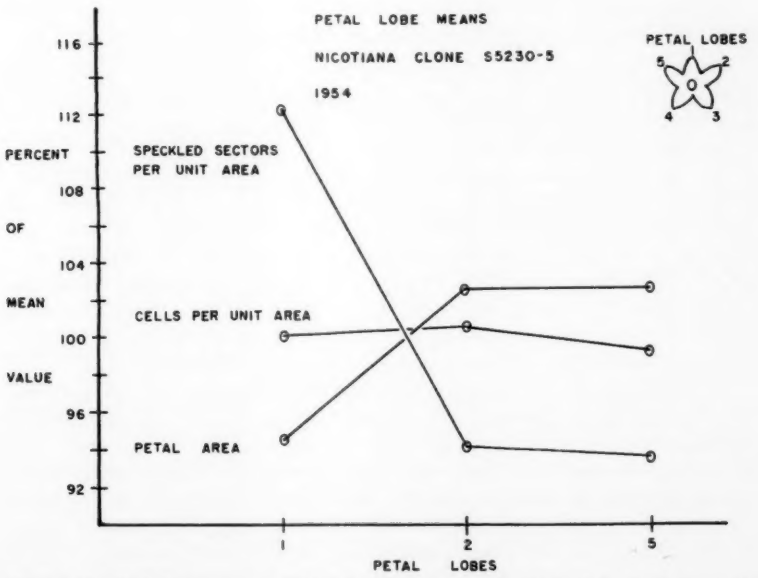

FIGURE 10.—Variation among petal lobes in the average values obtained for the three variates measured.

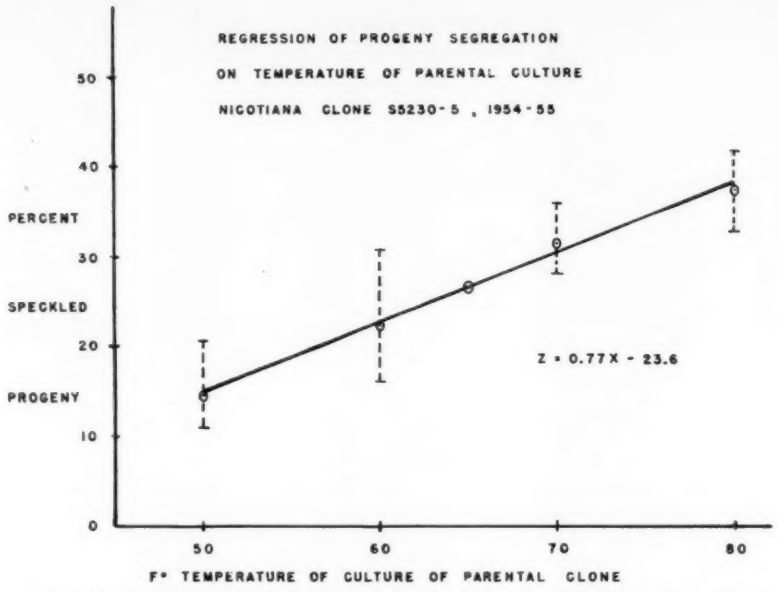

FIGURE 11.—Influence of the temperature at which the parental clone is cultured upon the frequency of speckled progeny obtained from these clonal parents.

regression of cell population density on temperature during progress of the experiment. This is also true for petal lobe width.

Further consideration of these results is given in the interpretation.

Effects of petal lobe position. The sketch accompanying the graphs of figure 10 shows the convention which was used in designating petal lobes 1, 2, and 5. The graphs display the variation among the different lobe means for speckled sector frequency, cell population density, and petal area. There are more speckled sectors per unit area in lobe 1 than in lobes 2 or 5. Lobe 1 is smaller than lobes 2 or 5. However, the three lobes are not greatly different in their average number of epidermal cells per unit area. The statistical significance of the foregoing observations is shown in table 3. Their implications are discussed in a following section.

Effect of temperature on progeny segregation. While the data were being taken on the somatic sectoring of members of the clone, other flowers of the same plants were self-pollinated to produce seed at each temperature. Seed from several capsules was bulked for each plant, and these seed samples and the resulting seedlings were treated alike. Progeny families from two plants at each temperature were scored for their frequency of speckled individuals. The results for 342 total offspring are summarized in figure 11. The progeny segregations are different from seed set at the different temperatures, giving higher frequencies of speckled offspring from the higher temperature treatments.

Interpretation of temperature effect studies

No estimate of a possible plant by treatment interaction is provided because each cutting was subjected only to one temperature treatment within the time period of the experiment. Thus the interpretation is based upon the assumption that plant randomization was adequately successful in distributing any plant by treatment interactions without order over the different treatments.

Joint consideration of treatment and order effects is essential because of the clear pattern of interaction between these two effects on speckled sector frequency. Figure 8 dramatizes the pattern of this interaction and tables 3 and 4 demonstrate its statistical significance. It is clear that the temperature effect on speckled sectors per square centimeter increases with the passage of time following introduction of the plant to the different temperature treatments. It ranges in an orderly manner from an average *decrease* of 5.7 percent of the mean per three orders of harvest at 50°F through *decreases* of 4.8 and 3.2 percent at 60 and 70 degrees to an average *increase* of 16.4 percent of the mean per three orders at 80°F.

There are two effects confounded in orders: time order of harvest and order of bloom or position on the branch. The analysis would be clear if it could be assumed that the orders of harvest would have been homogeneous in the absence of different temperature treatments. However, the data favor an increase in sector frequency as the rate of growth of the plant increases. This is indicated for the early period of growth of each branch and is suggested also by the increased sector frequency accompanying increased rate of bloom at higher temperatures. If there were such a correlation between sector frequency and flower position on the branch, it would appear that this increase had been overcome and thrown into a net decrease with the passage of time by larger temperature induced decreases at 50, 60, and 70 degrees F. Whereas one would interpret that this node-position increase had been augmented by culture at 80°F, thus accounting for the divergence noted between the 70 and 80 degree regressions cited above.

However, it should be emphasized that despite the compound nature of the order effect, the pattern of its interaction with temperature treatments favors the interpretation that temperature affects the number of sectors scored per square centimeter.

But since the temperature of culture also affects the size of the epidermal cells and thus the number of cells in one square centimeter (cell population density) of petal area, it is important to determine if the number of sectors counted in these areas could be different merely because the areas contain different numbers of cells.

Figure 9 shows that the effect of temperature on cell population density is present very early in the experiment and that the slope of the regression does not differ greatly from one percent of the mean per F degree throughout the experiment. Apparently cell size responds quickly to temperature whereas sectors per square centimeter respond more slowly, as would be expected if the sectors represent cell lineages. It is evident that the temperature effect on speckled sectors per square centimeter reaches a value during the last third of the experiment which cannot be accounted for by the nearly constant regression on temperature of cell population density. This conclusion is supported by table 4, which demonstrates that speckled

sector frequency adjusted to the mean cell population density continues to show a regression on temperature.

Another approach is to compare the average error regression with the regression of treatment means, both of which are graphed in figure 7. The statistical significance of the difference between these two slopes is shown in table 4. Any factor which affects cell size is expected also to affect the number of sectors counted per square centimeter because of the correlated alteration of the number of cells sampled in one square centimeter of petal area. The magnitude of this source of technical correlation is estimated by the average error regression coefficient: a measure of the variation in sector frequency attributable to variation in cell size. A principal source of this variation in cell size within treatments is likely the variation in degree of maturity of bloom at harvest. If temperature were causing a variation in scored sector frequency merely because of an effect upon cell size, then the regression of treatment means should be the same as the average error regression. Only a controlled factor which affects sectoring frequency independently of cell size should give the observed regression differences. This is evidence in favor of a more primary effect of temperature upon sectoring frequency. Reservations are made, however, because the present studies have not eliminated the possibility that the detected temperature effect may be due to differential competition (dependent upon temperature) between epidermal cells following the primary mutational events.

Also, if increased temperature caused the mutational events to occur earlier in development, it could simulate increased frequency of somatic sectoring in these data because a lower size limit was put on the sectors scored. However, the progeny segregations (fig. 11) for which the sampling procedure does not eliminate products of late mutational events, indicate a temperature effect on frequency independent of timing. But this conclusion is justified only if it is assumed that the different segregations result primarily from temperature influence on gamete proportions of the treated clone, rather than from influence during embryo development of the seed progenies on the treated clone.

It is interesting to note from figure 6 the negative correlation between cell population density and petal area or lobe width. Table 4 demonstrates, however, that all of the increase in petal width accompanying decrease in temperature cannot be accounted for by the correlated increase in cell size. Apparently the longer period of floral development at lower temperatures results not only in larger individual cells but also in more cell divisions to give a larger total number of epidermal cells. Both mechanisms contribute to the larger flower size at 50°F than at the higher temperatures.

Figure 10 and table 3 show that lobe 1 gives a significantly higher speckled sector frequency than lobes 2 or 5. But this cannot be due to differences in cell population density for table 3 demonstrates that the petal lobes do not differ significantly in the latter variate. It should be noted, however, that petal area or width is less for lobe 1 than for lobes 2 or 5, as is shown in figure 10 and table 3. Thus it would appear to follow either: (1) that the intrinsic sectoring frequency of lobe 1 is greater than that of lobes 2 or 5, or (2) that a sampling problem results in more sectors being counted on the smaller lobe. The writer prefers the latter interpretation. Small sectors are more concentrated near the petal margins (see fig. 5) and the square centimeter scoring

area includes more of these marginal sectors when superposed on a small petal than it does when superposed on a large petal.

A similar interpretation cannot be invoked, however, to explain as illusory the measured temperature effect on sector frequency. In such a case the sector frequency response to temperature should be present as soon after initiating treatment as is the petal size response to temperature. The more delayed response observed for sector frequency indicates a component of the response that is influenced by temperature independently of petal size.

It should be noticed also that since the smaller size of lobe 1 is not accompanied by the presence of smaller cells it must be that it is due to fewer cells being present. Thus this inherent size difference in lobes is mediated by control of the number of cell divisions, whereas the temperature-induced size difference in petals has both a cell size and cell number component.

DISCUSSION

Genetic studies of the variegated-1 culture of Nicotiana plants (SMITH and SAND 1957) demonstrate that a single major locus, V, controls inheritance of the variegation system. These studies also favor the interpretation of segregation for two alleles, v_s and v_S, which undergo relatively frequent changes similar to reversible mutations. The reversals resemble temporary inhibition and release from inhibition of a locus critical to pigment synthesis.

Reversible changes in somatic tissue, with that from v_S to v_s occurring earlier in development than the reverse change, are consistent also with the variegated phenotypes. Thus, by this interpretation solid-colored tissue results from the presence of the locus in its uninhibited condition, v_S. A change at the locus to its inhibited condition, v_s, in certain cells during somatic development originates sectors of potentially colorless tissue. Such sectoring events occurring at an early stage in development result in larger sectors at maturity than do similar events occurring later in development. However, these sectors are not completely colorless, but contain clusters (and rarely small subsectors) of pigmented cells. This speckled condition is considered to be due to a reverse change at the locus from v_s to the uninhibited condition, v_S, in certain cells of the sector very late in development. Thus it is proposed that a study of phenotypic variability in these variegated flowers is a study of variation in the frequency and developmental timing of reversible genetic events which alter the function of the v locus in somatic tissue. These functional alterations may resemble the "local state" changes at the H_2 locus in Salmonella (LEDERBERG and IINO 1956).

CATCHESIDE (1947) has reported a variegated type position effect on sepal and petal color in Oenothera. Translocated dominant loci express themselves during development alternatively as normal dominant and lower grade mutant alleles. Although there is presently no direct evidence for the involvement of a translocation in the Nicotiana material, the alternating phenotypic expression in these variegated cultures resembles that in the Oenothera case.

DEMEREC (1931) studied the rates of mutation of two genes of *Delphinium ajacis*. In the case of the rose-alpha gene he found the rate of mutation to purple to be relatively constant in somatic tissue during different stages of sepal and raceme develop-

ment. Lavender-alpha had a high mutability early and late, and a low mutability in mid-stages of ontogeny. The variegated-1 (sectorial) phenotype resembles rose-alpha: the sector size and position distributions suggest that the sectors represent ontogenetic cell lineages and that the frequency of the change v_S to v_s is not greatly different during successive stages of development. Alternatively in the variegated-3 (flecked) phenotype the change v_S to v_s is largely confined to relatively late stages of petal development. This difference in developmental timing of the sectoring event is heritable, but its genetic basis is not yet clear.

In this connection it should be observed that McCLINTOCK (1951) has shown a genetic control of the timing of mutational events in maize endosperm. In her *Ds-Ac* system an increase in dosage of *Ac* results in later developmental timing of *Ds* events.

Variegation in the Nicotiana cultures appears to result from reversible changes in the functional condition of a genetic locus. The developmental timing and the frequency of these changes are subject to genetic control, but are also critically influenced by environmental temperature.

Environmental influence on variegation in maize has been shown by EYSTER (1926) and by VAN SCHAIK (1955). LEWIS (1953) has demonstrated effects of environmental factors including temperature upon the frequency of rogue tomatoes. Specific temperature effects on Drosophila mosaicism have been shown by PROKOFYEVA-BELGOVSKAYA (1947) and by HOUSE (1955). And MULLER (1928) detected a temperature effect upon the frequency of lethals in Drosophila. In maize possessing the gene *Dt*, RHOADES (1941) demonstrated a temperature effect upon the frequency of mutations from *a* to *A*. PETERSON (1952) has reported a temperature influence upon a mutable pale green locus in maize, and FABERGÉ and BEALE (1942) found an effect of temperature on an unstable gene in Portulaca. The direction of the effect has varied with the material employed in these cases of temperature influence on genic expression or mutation. Similarly in the Nicotiana material, the effect of higher temperature is to increase the rate of change from v_S to v_s but to decrease the reverse change.

SUMMARY

Nicotiana cultures are employed in which variegation of flower color is interpreted in terms of reversible "mutations" between the allelic forms v_s and v_S. Differences in frequency and developmental timing of these events have both heritable and environmental components. Several causes of the resulting phenotypic variability are evaluated.

Although speckling intensity increases with age of the flower, different speckled plants are shown to vary in their average frequencies of pigmented epidermal cells. This reflects differences in their rates of somatic change from v_s to v_S, which are consistent with their different breeding behaviors. Similarly, different sectorial plants vary phenotypically in their average frequencies of sectors, thus showing different rates of somatic change from v_S to v_s, which are correlated with different breeding behaviors.

Data are presented which indicate that higher temperatures decrease somatic

speckling but increase sectoring. And for the latter phenomenon a resulting effect on progeny segregation has been detected.

Higher temperatures during development also result in a decreased epidermal cell size and a decreased petal size. Consideration of these variables shows that the temperature treatments give different sectoring frequencies per unit cell population. In addition these data indicate that the temperature induced differences in petal size have both a cell size and a cell number component, whereas the inherent difference in size between particular petal lobes of the same flower probably is due only to cell number.

The distribution of sector size favors that the sectors delineate ontogenetic cell lineages for the petal epidermis.

The distribution of sector position is evidence that these sympetalous Nicotiana flowers are developmentally polypetalous.

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THE RELATIONSHIP OF GENES FOR PATHOGENICITY AND CERTAIN OTHER CHARACTERS IN VENTURIA INAEQUALIS (CKE.) WINT.[1]

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VENTURIA inaequalis (Cke.) Wint., the ascomycetous pathogen of apple scab, has been shown to have adaptations similar to the eight-spored Neurospora species for use in genetic analyses (KEITT and LANGFORD 1941; SHAY and KEITT 1945; KEITT *et al.* 1948; BOONE and KEITT 1956). It has, in addition, two very important advantages over Neurospora: 1) It is pathogenic and can be studied *in vivo*; and 2) it is uninucleate in its vegetative stage and, thus, is free from the confounding effects of heterocaryosis. Pathogenicity in *V. inaequalis* is variable and each instance of virulence that has been studied adequately has been shown to be controlled primarily by a single gene (KEITT 1952; SHAY and HOUGH 1952; SHAY and WILLIAMS 1956). Many physiological races could be recognized on the basis of pathogenicity on apple varieties, but, so far, there has been no advantage in naming more than three (SHAY and WILLIAMS 1956). The purpose of this paper is to present the relationships of the pathogenicity genes differentiating the three recorded races of the organism. A preliminary account has been published (SHAY and WILLIAMS 1953).

MATERIALS AND METHODS

The Host: All pathogenicity determinations were made on clonal selections of apple in the greenhouse during the months of February, March and April in the years 1953–56. These selections include the varieties Dolgo and Geneva, a selection (MA 1363-24) of *Malus sikkimensis*, and four seedlings, OR42T141, OR42T142, OR42T145, and OR42T173. These seedlings were selected for their uniform susceptibility to a race of the pathogen and are of the parentage, McIntosh × (R12740-7A × Delicious). Since these seedlings gave similar disease reactions in all tests, they are grouped together in this paper under the name "Russian", to denote the parent, R12740-7A, a seedling received from Russia (DAYTON *et al.* 1953).

The selections included in this study have been classified by SHAY and HOUGH (1952) and SHAY and WILLIAMS (1956) with respect to leaf symptoms incited by isolates carrying the pathogenicity factors under consideration. These reaction classes are as follows: 1 = numerous or few minute pits, no sporulation; 2 = irregular or regular necrotic or chlorotic lesions, no sporulation; 3 = restricted necrotic lesions with sporulation, no defoliation; 4 = extensive lesions with abundant sporulation, leaves may abscise.

The Pathogen—Description of Characters: The pathogenicity factors under consideration were found by SHAY and HOUGH (1952) and SHAY and WILLIAMS (1956) to be controlled by single genes in the fungus. On the basis of these results, the sym-

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bols listed below are assigned to these factors. This method of classifying and numbering the pathogenicity genes in *Venturia inaequalis* was established in conjunction with DRS. BOONE and KEITT of the University of Wisconsin. Through mutual agreement, the symbols *p-1*$^+$ through *p-7*$^+$ were reserved for genes controlling pathogenicity on commercial varieties under observation at Wisconsin. In this system of classification, the suffix "+" is used to denote full virulence on the stated variety. In some cases, this is the commonly occurring type and in others it is rare or not yet obtained.

p-8$^+$/*p-8*: *p-8*$^+$ conditions the 3 reaction class on Dolgo (Plate 1, B); while the allele, *p-8*, incites the 2 reaction class (Plate 1, A). It was discovered in the course of the investigation that another gene (unidentified) in the fungus intensifies the effect of *p-8*$^+$, conditioning a 4 reaction on Dolgo. As the effect of *p-8*$^+$ is always expressed and the intensifier gene is not, only this gene is considered in the present study.

p-9$^+$/*p-9*: *p-9*$^+$ conditions the 4 reaction class on Russian (Plate 1, D); while the allele, *p-9*, conditions the 2 reaction class (Plate 1, C).

p-10$^+$/*p-10*: *p-10*$^+$ conditions the 4 reaction class on Geneva (Plate 1, G) while the allele, *p-10*, conditions the 2 reaction class (Plate 1, E).

p-11$^+$/*p-11*: *p-11*$^+$ conditions the 3 reaction class on Geneva (Plate 1, F) while the allele, *p-11*, conditions the 2 reaction class.

p-12/*p-12*^{1}: *p-12*^{1} incites the 1 reaction class (Pinpoint pits, Plate 1, H) on *Malus sikkimensis*; while the allele, *p-12*, incites large necrotic lesions (2 reaction class, Plate 1, I) with tan centers and red-brown margins on this selection. (The genotypes *p-12*$^+$ and *p-13*$^+$ have not been discovered.)

p-13/*p-13*^{1}: *p-13*^{1} incites pinpoint pits with slight necrosis in the base on *M. sikkimensis*; while the allele, *p-13*, incites the typical 2 reaction class described above.

In addition to the pathogenicity genes described above, two additional genes, green and mating type, were included. These genes were used as aids in identifying ascospore pairs. The position of one, green, is known (BOONE and KEITT 1956).

green (*gr*$^+$/*gr*): *gr* is characterized by a bottle green mycelial color; while the allele, *gr*$^+$, present in wild type isolates, is characterized by the typical brown-olivaceous mycelial color.

mating type (*mt*$^+$/*mt*$^-$): *mt*$^+$ was arbitrarily assigned to the type of standard isolate 356-2; while *mt*$^-$ was assigned to the type of standard tester isolate 651.

Cultural Methods: Cultures were maintained under sterile mineral oil on potato dextrose agar (PDA) in tubes. *In vitro* matings of the isolates and isolation of the ascospores were made manually with a glass needle by the method of KEITT and LANGFORD (1941). After isolation and when sufficient growth had developed, the ascospore cultures were numbered and classified according to ascospore pairs. Some transposition of spores in the ascus was observed. Only asci in which the original position of the spores could be deduced were included in the classification of segregation of factors.

To determine mating types, crosses were made between each of the ascospore isolates and one or both of the mating type tester isolates, 356-2 and 651.

Inoculation Methods: Inoculum was produced by the method of KEITT and PAL-

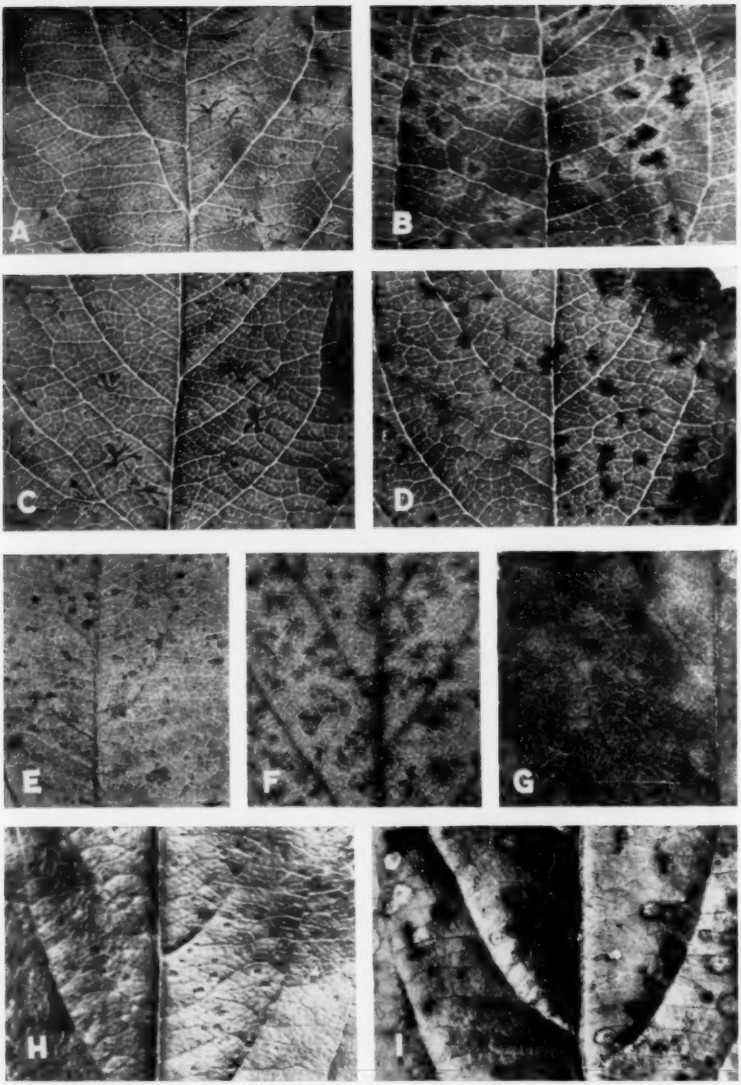

PLATE 1:—A. Reaction class 2 incited on Dolgo by isolates with genotype *p-8*. B. Reaction class 3 incited on Dolgo by isolates with genotype *p-8*$^{+}$. C. Reaction class 2 incited on Russian differentials by isolates with genotype *p-9*. D. Reaction class 4 incited on Russian differentials by isolates with genotype *p-9*$^{+}$. E. Reaction class 2 incited on Geneva by isolates with genotype *p-10* and/or *p-11*. F. Reaction class 3 incited on Geneva by isolates with genotype *p-11*$^{+}$. G. Reaction class 4 incited on Geneva by isolates with genotype *p-10*$^{+}$. H. Reaction class 1 incited on *M. sikkimensis* by isolates with the genotypes *p-12*^{1} and/or *p-13*^{1}. I. Reaction class 2 incited on *M. sikkimensis* by isolates with the genotypes *p-12* and/or *p-13*.

MITER (1938) modified to the extent that cheesecloth wicks in 4 ounce prescription bottles were used. Inoculum was diluted to provide approximately 100 conidia per low power (100×) microscope field and was applied to the terminal leaves of the plant by means of a number 15 DeVilbis atomizer attached to an airline providing 15 pounds pressure. The inoculations were made in a plastic covered chamber in a room removed from the greenhouse. Between inoculations, the interior was washed down with a water spray. The inoculated plants were kept for 44 hours in a moist chamber consisting of a frame covered with wet muslin. Infection data were taken two weeks after inoculation. The plants were then returned to the moist-chamber for another 44 hours to enhance maximum symptom development. Readings were taken again immediately upon removal.

Methods Used for Computing Map Distances: The location of genes with respect to centromere was calculated by the method developed by LINDEGREN (1933) based on the percent of asci showing second division segregation of the gene-pair (alleles) concerned.

Tests for homogeneity of data were made in the manner of BARRATT and GARNJOBST (1949). Using the percent of second division segregation of all crosses for a single factor as a base, Chi square values were calculated for each cross. These values were totaled and P was calculated for the total Chi square value.

The tests for independence of factors were based on the hypothesis that the factors are inherited independently of each other. Chi square values were calculated for the ratio of parental and nonparental ditype asci. As pointed out by PERKINS (1953) values obtained by this method are not obscured by excessive numbers of tetratype asci.

No attempts were made to correct for multiple crossovers or for possible chiasma or chromatid interference.

The limits in which the true values of the map distance might be expected to lie were determined from the 95 percent confidence limit curves of BARRATT, *et al.* (1954, Fig. 8).

TABLE 1

Summary of the data on segregation of the gene pairs included in this investigation

Gene symbols	Total asci no.	Second division segregation		Test for homogeneity of data*			Distance from centromere	95% confidence range†
		No.	Percent	df	x^2	P		
p-8$^+$/*p-8*	316	211	66.8	17	15.8	0.54	—	30.7 to —
p-9$^+$/*p-9*	191	122	63.9	12	13.7	0.33	—	28.5 to —
p-10$^+$/*p-10*	123	78	63.4	10	5.6	0.85	—	27.0 to —
p-11$^+$/*p-11*	78	46	59.0	3	3.6	0.32	—	22.5 to —
p-12/*p-12*^{1}	33	15	45.5	4	3.1	0.55	22.7	13.8 to 31.7
p-13/*p-13*^{1}	24	17	70.8	3	3.5	0.33	—	27.0 to —
mt$^+$/*mt*$^-$	289	187	64.7	18	9.4	0.95	—	27.0 to —
gr$^+$/*gr*	312	215	68.9	12	8.4	0.75	—	31.5 to —

* Tests for homogeneity made in the manner of BARRATT and GARNJOBST (1949).

† Based on 95 percent confidence limit curve of BARRATT *et al.* (1954, Fig. 8).

RESULTS

Gene to Centromere Distance: Chi square tests for homogeneity for each factor indicate that the data for all crosses involving a particular factor are homogeneous and can be combined. Presented in table 1 is a summary of the data relating to the location of these factors with relation to their centromeres as determined by LINDEGREN'S (1933) method. All of the genes under consideration are distant from their centromeres and only one, *p-12*^{1}, deviates significantly from random assortment and, on the basis of these data, is located at 22.7 crossover units from its centromere. The 95 percent confidence range limits calculated for this gene indicate that *p-12*^{1} apparently lies at a point between 13.8 and 31.7 units from the centromere.

On the basis of percent of second division segregation of the factors controlling pathogenicity on Geneva, *p-10*$^+$ and *p-11*$^+$ might be judged to be allelic. However, progeny recombinations from crosses involving these two genes (see table 2) indicate that they are not allelic and apparently not even linked. The relationship of *p-12*^{1} and *p-13*^{1} is less distinct. Crosses involving the two genes have not been available. However, as the recombination data between these genes and *p-8*$^+$ (see table 2) indicate a marked difference, *p-12*^{1} and *p-13*^{1} are considered, tentatively, as being nonallelic.

TABLE 2

Summary of the data on recombination of genes for pathogenicity and certain other characters

Segregating characters*	Total no. asci	Observed tetrad numbers†			Test for independence‡ P	Percent recombination	Conclusions
		PD	NPD	T			
p-8$^+$, *p-9*$^+$	173	130	0	43	<0.01	12.4	Linked
p-8$^+$, *p-10*$^+$	97	21	15	61	0.32	46.9	Independent
p-8$^+$, *mt*$^+$	234	31	33	170	0.89	50.4	Independent
p-8$^+$, *gr*	181	33	23	125	0.19	47.2	Independent
p-8$^+$, *p-12*^{1}	32	16	0	16	<0.01	25.0	Linked
p-8$^+$, *p-13*^{1}	23	4	4	15	0.99	50.0	Independent
p-8$^+$, *p-11*$^+$	71	15	8	48	0.15	45.1	Independent
p-9$^+$, *mt*$^+$	168	26	25	117	0.89	49.7	Independent
p-9$^+$, *p-10*$^+$	76	13	8	55	0.27	46.7	Independent
p-9$^+$, *p-12*^{1}	29	9	2	18	0.04	37.9	Linked
p-9$^+$, *gr*	116	22	13	81	0.13	46.1	Independent
p-9$^+$, *p-11*$^+$	80	15	7	58	0.09	45.0	Independent
p-10$^+$, *p-12*^{1}	12	2	0	10	0.16	41.7	Independent
p-10$^+$, *mt*$^+$	89	13	13	63	0.99	50.0	Independent
p-10$^+$, *gr*	52	12	10	30	0.68	48.1	Independent
p-10$^+$, *p-11*$^+$	25	5	8	12	0.42	56.0	Independent
p-11$^+$, *gr*	78	10	11	57	0.84	50.6	Independent
p-11$^+$, *mt*$^+$	78	13	10	55	0.54	48.1	Independent
p-12^1, *mt*$^+$	28	4	7	17	0.38	55.4	Independent
p-13^1, *gr*	24	3	8	13	0.14	60.4	Independent
p-13^1, *mt*$^+$	15	3	0	12	0.09	40.0	Independent
gr, *mt*$^+$	147	27	26	94	0.89	49.7	Independent

* See text for description of characters.

† PD = parental ditype asci; NPD = non-parental asci; and T = tetratype asci.

‡ Test for independence based on PD:NPD deviation from 1:1.

TABLE 3

Linkage Group A

Cross	Number asci tested	Gene to gene distance*	95% Confidence range limits†
p-8$^+$, *p-9*$^+$ × *p-8*, *p-9*	173	12.4	9.0 to 15.0
p-8$^+$, *p-12* × *p-8*, *p-12*^{1}	32	25.0	15.5 to 34.0
p-9$^+$, *p-12* × *p-9*, *p-12*^{1}	29	31.0	21.9 to 40.5

* Corrected distance based on the method of BARRATT *et al.* (1954) in which one half the percentage of tetratype asci is taken as the uncorrected distance in crossover units between the genes.

† Based on BARRATT *et al.* (1954, Fig. 8) method of determining 95 percent confidence range interval.

The factor green has been included in BOONE and KEITT's (1956) inheritance studies with color mutants. Through linkage studies, these workers have located this gene at a point between 45 and 55 crossover units from the centromere.

Gene to Gene Distance: The results of the 22 combinations of characters studied are listed in table 2. The tests for independence, made in the manner suggested by PERKINS (1953) based on parental ditype:nonparental ditype ratios, indicate that *p-8*$^+$ is linked with *p-12*^{1} and *p-9*$^+$. All other combinations studied were not significantly different from a 1:1 ratio and can be considered as not linked. Six gene pair combinations were not tested. These include *p-13*^{1} with *p-9*$^+$, *p-10*$^+$, *p-11*$^+$ and *p-12*^{1}; and *p-12*^{1} with *p-11*$^+$ and *gr*. As *p-12*^{1} can be located with respect to its centromere (see table 1) and with the genes, *p-8*$^+$ and *p-9*$^+$, and as *p-8*$^+$ and *p-9*$^+$ have been shown to be inherited independently of *p-11*$^+$ and *gr*, it can be assumed that the latter genes are not linked with *p-12*^{1}.

The linkage group formed by genes *p-12*^{1}, *p-8*$^+$ and *p-9*$^+$ is designated as Linkage Group A (see table 3) until it is determined where it fits in BOONE and KEITT's (1956) gene linkage classification. Based on the method of computing corrected gene to gene distance as one half the percentage of tetratype asci (BARRATT *et al.* 1954), *p-12*^{1} and *p-8*$^+$ are placed at 25 crossover units apart; *p-8*$^+$ and *p-9*$^+$ at approximately 12 units; and *p-12*^{1} and *p-9*$^+$ at 31 units. These distances are uncorrected for multiple crossovers. While the number of asci included in this test are not large, these data indicate that the *p-12*^{1}, *p-8*$^+$, and *p-9*$^+$ are arranged on the chromosome in that order with *p-12*^{1} being nearest to the centromere.

DISCUSSION

It has been pointed out by BLACK (1952) that the identification and determination of the relationship of genes for pathogenicity in a microorganism are important not only from the standpoint of use in a breeding program but also as an aid in determining biosynthetic pathways and ultimately in working out the nature of resistance in the host.

The study of host-parasite relationship is complicated with pathogens such as rusts and smuts. This is in part due to the dicaryotic condition of nuclei in the vegetative stage. However, *Venturia inaequalis*, with its uninucleate vegetative cells, is singularly adapted for use in such a detailed study. This character, in addition to suitability of the host for asexual propagation affording a continued supply of genet-

ically identical host material, presents a favorable basis for future studies on host-parasite interaction.

Pathogenicity in *V. inaequalis* has been shown to be controlled primarily by a single gene (KEITT 1952; SHAY and WILLIAMS 1956). Resistance in the host has not been completely worked out. However, there are indications that, with the host material included in this study, resistance may be controlled by single genes (SHAY *et al.* 1953; DAYTON *et al.* 1953).

The pathogenicity gene symbol classification employed in this study is designed to facilitate the orderly presentation of data as further studies with this organism are completed. Furthermore, for the same reason, it is believed that a comparable method of naming genes for resistance in the host would be advantageous. For instance, these genes can be identified as R_1, R_2, R_3, etc., with each differential resistance gene being given the corresponding pathogenicity number as it is isolated in the gene population.

Based on this system of pathogenicity gene classification, isolates of the races described by SHAY and WILLIAMS (1956) can be assigned the following pathogenicity genotypes: *Race 1* isolates are nonvirulent on Dolgo, Russian, and Geneva, and, thus, would have the genotype *p-8*, *p-9*, *p-10*, *p-11*; *Race 2* isolates are virulent on the three varieties and would have the genotype *p-8*$^+$, *p-9*$^+$, *p-10*, *p-11*$^+$; while *Race 3* isolates are virulent only on Geneva and have the genotype *p-8*, *p-9*, *p-10*$^+$, *p-11*.

It is of interest that all pathogenicity genes under consideration are located at considerable distances from their respective centromeres. KEITT and BOONE (1952) have described six gene pairs conditioning pathogenicity on commercial varieties of Malus. Of these, only one, Haralson$_2$, was found to be close enough to the centromere to determine map distance by second division segregation. This gene and the gene, *p-12*^{1}, conditioning pin-point pits on *M. sikkimensis*, are approximately the same distance from their respective centromeres.

SUMMARY

The four factors controlling pathogenicity in the three named races of *Venturia inaequalis*, two factors conditioning reduced virulence against *Malus sikkimensis*, and single factors controlling green mycelial color and sex compatibility in the fungus, were studied with respect to linkage relationship and centromere distance. Three factors, *p-12*^{1}, conditioning reduced virulence symptoms on *M. sikkimensis*, *p-8*$^+$, conditioning pathogenicity against *M. baccata* var. Dolgo, *p-9*$^+$ conditioning pathogenicity against a differential selection of *M. pumila* # R12740-7A, and the centromere were found to be loosely linked. All other factors were inherited independently of each other and of their respective centromeres.

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EGG MORTALITY AND INTERCHROMOSOMAL EFFECTS ON RECOMBINATION[1]

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IN *Drosophila melanogaster* appreciable increase in recombination may be produced for a given pair of chromosomes by the introduction of heterozygous inversions into other chromosome pairs. (A general review of the evidence has been published by SCHULTZ and REDFIELD 1951; details are given in papers mentioned there and in those quoted below; recent examples of similar effects in other Drosophila species may be found in the work of CARSON 1953, and LEVINE and LEVINE 1954.) The effects vary with the inversion set-up but they fall into a general pattern showing relatively large increments near the centromere and typically also at free ends of chromosome limbs; less marked increments are found in intermediate regions. These increases are striking and have been emphasized in the literature, but it must not be forgotten that decreases also are occasionally demonstrated. Compared with the increases, the decreases are quantitatively quite small, but they do appear to be real (MORGAN, REDFIELD and MORGAN 1943; STEINBERG and FRASER 1944; WELSHONS 1955.) But after recognizing these exceptions it is still desirable to focus attention primarily on the increases, since the positive effect produced by the latter is of a larger order of magnitude than is the negative effect of the relatively quite minor and definitely restricted decreases.

The basic cause of this influence of heterozygous inversions upon crossing over in "independent" chromosome pairs has remained obscure, although there is an obvious resemblance of the pattern of increase to the gross distribution pattern of heterochromatin along the chromosome limbs. Of probable relevance is the cytological study of SCHULTZ and HUNGERFORD (1953; their paper presented at the 1952 meeting of the Genetics Society of America was kindly made available) which shows that the pattern of somatic pairing between X chromosomes is significantly changed in the presence of heterozygous inversions in chromosomes II and III. This change accompanies the increases in crossing over demonstrated at the same time by REDFIELD (1953, 1955) for the X chromosomes of the same material.

Since the 1916 paper of BRIDGES it has been recognized that a curious relationship exists between crossing over and disjunction of the X chromosome, for in the exceptions resulting from secondary nondisjunction those X strands which emerge are noncrossovers. More recently STURTEVANT has briefly reported (MORGAN and STURTEVANT 1944) that heterozygous autosomal inversions which decrease secondary X nondisjunction, may increase primary X nondisjunction. Following this lead from primary nondisjunction COOPER, ZIMMERING and KRIVSHENKO (1955) suggest

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for complex structural heterozygotes an hypothesis involving pairing of non-homologues from two or more chromosome pairs, with subsequent nondisjunction of autosomes (as well as of X chromosomes), and with selective elimination in unhatched eggs of strands of lower rank crossover types. This they believe "will partially account for an interchromosomal effect on crossing over". Obviously such a series of assumed events might, if actually shown to be of sufficient frequency, be a factor of real significance in explaining the crossover increases with heterologous inversions. It seems advisable therefore to consider the expected consequences if elimination of noncrossover strands were to have a *major* role in the production of the observed increases—but, of course, COOPER, ZIMMERING and KRIVSHENKO are not to be held responsible for this extended use of their suggestion.

Increased death of eggs among offspring of inversion heterozygotes is a matter of common observation, and it has previously been explained (STURTEVANT and BEADLE 1936; CARSON 1946, 1953) as the result of the production of certain expected aberrant strands by exchanges between homologues, and within inverted sections. An alternative, or accessory, explanation of the egg mortality obviously exists if there is a marked amount of non-homologous pairing of chromosomes, for such would be expected to lead to considerable nondisjunction of major autosomes, and thus to appreciable production of lethal zygotes with an excess or a deficiency in the normal complement of these autosomes. This matter will be dealt with again later; but it may be briefly noted here that although such eggs might conceivably be assumed to carry with them to early destruction noncrossover strands of the heterozygous inversion pairs, it is not immediately clear that noncrossover strands of remaining chromosome pairs, that is those without inversions, would be selectively eliminated. And of course just this latter situation, in which heterologous inversions affect recombination in remaining structurally like pairs, is the critical test of the interchromosomal effect of inversions.

But the main concern of the present paper is to determine whether the amount of egg mortality actually produced by selected heterologous inversions under optimal experimental conditions can, in terms of elimination of noncrossovers, account statistically for the observed recombination increases given by the inversions. Recombination effects, shown by X chromosomes structurally alike, will be compared with egg mortality results. For this the relatively simple Curly and Payne inversions of chromosomes II and III, respectively, were used. These inversions were chosen, not only because they have figured prominently in establishing the interchromosomal effects on recombination, but primarily because as inversions go they have little effect on viability—and incidentally because they do not, unlike some inversions, include translocations which definitely complicate matters through independent production of autosomal nondisjunction.

RESULTS

The simple egg counts

The preliminary egg counts came from females of the "p-26" stock of this laboratory; the stock contains the left and right Curly inversions of II, and the left and right Payne inversions of III; the particular Payne chromosome used includes the

identifying dominant Deformed eye (*Dfd*). (For a description of these inversions see BRIDGES and BREHME 1944.) To guard against loss of the inversions by crossing over within the stock, the Curly and Payne chromosomes are continued in each generation through a single male parent, the single female parent being introduced as a wild type virgin from "p-1 Oregon-R". (At the time, this latter stock had been inbred some 135 generations by individual brother-sister matings.) Continued introduction of inversions from the father is generally advisable for the stable maintenance of a number of inversions in a single stock; this has become evident through occasional experimental fiascoes shown to be dependent on loss of inverted limbs through the increased crossing over taking place, even in the inverted chromosomes, in females with multiple inversions. A final check of the proper presence of the inversions was provided by cytological examination of the salivary gland chromosomes by DR. JACK SCHULTZ. There remains the question of variation in the noninverted chromosomes; but since all chromosomes of p-26, other than inversion chromosomes, are from the highly inbred p-1, the noninverted chromosomes of the different types are within all reasonable limits the same and isogenic with those of p-1.

Three crosses were involved in this first experiment; for each of these, virgins were isolated and mated in vials in small numbers (ordinarily five per vial) to three times their number of males. After a period of two to three days in the vials to insure proper mating, the flies of each cross were put into a single bottle, with 20 to 30 females per bottle. Twice daily, metal trays were provided with a layer of standard molasses-agar-Moldex medium the surface of which was scored by a needle into conveniently small rectangles, and painted with a solution of fresh yeast. Thus the first count of the eggs was made either early in the morning or late in the afternoon; then allowing from this count an interval of 24 hours for hatching, a second count was made of the eggs which did not hatch. Actual counts took place at room temperature—otherwise development of both parents and offspring was at a controlled 25°C. No eggs were collected after the females were 7 days old. The chances are believed good that with this technique all females are properly mated, and that all eggs are fertilized.

Data from the three crosses are shown in table 1; the results of the counts are clear-cut. Since the genetic constitution is controlled, the differences can be attributed to the presence of inversions in the mothers. Both the mating of wild type females by wild type males, and that of sister wild type females by brother *Cy*/+; *Payne*/+ males, give quite the same result—namely an 88 percent hatch of the eggs laid. Thus there is in each case a 12 percent loss by death in the egg stage, which may be regarded as a measure of the innate genetic weakness of the eggs of this particular stock. There is in these crosses no loss attributable to the weakening of the egg stage by any combination of the inversion chromosomes. A significant point for our purposes is that no nondisjunction of major autosomes has here been produced in the fathers by the presence of the inversions, since such would have given additions or losses transmitted by the sperm and would be detected as elimination of eggs in the one cross but not in the other.

Now the data show that the third cross, from the same culture bottles of *Cy*/+; *Payne*/+ females by wild type males gives a much higher egg loss: 78.6 percent of the eggs hatch, that is 21.4 percent die—this death rate is then approximately ten

TABLE 1

Egg counts from highly inbred stock. Inversions were introduced as indicated

Inversions			
Mothers	None	None	*Cy; Payne, Dfd*
Fathers	None	*Cy; Payne, Dfd*	None
Eggs laid	7450	5043	5093
Eggs unhatched	11.7%	11.8%	21.4%

percent greater than that shown by the other two crosses. There seems to be no immediate reason for attributing this observed increase of ten percent to nondisjunction of II and III since, as explained in the paragraph above, it does not occur in the reciprocal cross where the inversions are introduced from the fathers. It is well known (from work of MULLER, DOBZHANSKY, and of others) that nondisjunction of II and III, in those cases where it does with certainty occur, as in the presence of translocations, is found in both sexes, There is no justification for assuming that inversions would limit any nondisjunction they might cause to one sex only. However even if we were completely to ignore the significance of the demonstrated lack of autosomal nondisjunction in the inversion male, and were to assume all this ten percent difference really to be the product of nondisjunction of autosomes in the inversion female, this actual value is still far from sufficient statistically to account for observed recombination increases produced by these inversions in other crosses. This matter will be dealt with later, as will also the matter of the more probable explanation of the difference as the result of four strand double crossing over within inversions.

Egg counts accompanied by crossover counts

Evidence of a direct nature concerning a possible relationship between egg mortality and the recombination increases might be expected if crossover counts for the X chromosome were made for both *Cy+; Payne/+* mothers and for mothers without inversions, of the F_1 adults actually developing from survivors of egg counts. But in such an experiment one must, as will become evident, guard against complications resulting from additional lethal effects at the egg stage due, for example, to the particular mutants introduced to measure the crossing over.

A relatively simple cross was planned making use of the left portion of the X, for which region marked recombination increases are known to be produced by the Curly and Payne inversions of stock p-26 (REDFIELD 1955). Three additional stocks were utilized here which had been derived about a year previously by crosses such that all chromosomes were necessarily from p-1 with the possible exception of chromosome IV, and excepting in the X the yellow-split region and also small regions surrounding the mutant loci crossveinless and vermilion. These stocks had been carried as small mass cultures since their origin, and consequently cannot be regarded as genetically pure in the sense that p-26 is pure. Females of the composition $y^2 w^a$ *spl v/cv v* were obtained, with and without the inversions, and were crossed to males from the tester stock with all the recessive mutant genes except vermilion, and with the additional recessive suppressor of apricot. These genes have the following loci on the standard map: y^2, yellow^2, 0.0; w^a, apricot, 1.5; *spl*, split, 3.0; *cv*, crossveinless, 13.7;

v, vermilion, 33.0 (BRIDGES and BREHME 1944). *Su-w*^{a}, suppressor of apricot, is between the loci for *y* and *w* according to information supplied by DR. JACK SCHULTZ. It was hoped to obtain some incidental measure of primary nondisjunction of the X; this may be detected in female offspring of this cross by the presence of vermilion, and in male offspring by the presence of the suppressor of apricot which unmistakably darkens the apricot eye. The technique for egg collection did not differ from that described in the section above except that the number of males was four times the number of females instead of three times that number. For the crossover counts sample blocks of the medium from the egg collecting trays, each block containing approximately 100 larvae, were after the second egg count, placed separately in ordinary culture bottles where they developed into adults. It was not considered feasible to allow all surviving eggs to develop, and excess blocks were discarded. No accurate count was attempted of larvae or of pupae, and it is not known precisely what the mortality was at these stages; nevertheless dead larvae of late stages and dead pupae were certainly not present in large numbers.

Table 2 gives the data for egg counts and for crossover counts. The picture presented by the egg counts is somewhat different from that shown by the results of the preceding section, for there is here a much more marked difference between the percentages of unhatched eggs obtained from the two types of mother. Mothers without inversions (table 2) show a lower percentage of egg lethals, 3.2 percent, than do comparable mothers of the p-26 stock shown in table 1 which gave 11.7 percent. On the other hand inversions mothers (table 2) show 26 percent of egg lethals, whereas those in table 1 show 21.4 percent. Thus the percentage of so-called "dominant lethals" due to the presence in the mothers of the inversions is approximately ten percent (21.4 percent minus 11.7 percent) for p-26 stock, but it is approximately 23 percent

TABLE 2

Egg counts and recombination counts from y^2 w^a spl + v/+++ cv v ♀♀ × y^2 su-w^a w^a spl cv ♂♂. *The inversions are introduced as indicated*

Inversions		
Mothers	None	*Cy; Payne, Dfd*
Fathers	None	None
Eggs laid	8824	8317
Eggs unhatched	3.2%	26.0%
Crossover type		
0	2218 + 2178	1238 + 831
1	21 + 23	67 + 83
2	17 + 17	34 + 42
3	187 + 171	290 + 347
1,3	1 + 0	3 + 4
2,3	0 + 0	1 + 0
N	4833	2940
X nondisjunction	0.06%	0.17%
Crossover values		
y^2 – w^a	0.93 ± 0.14	5.34 ± 0.41
w^a – *spl*	0.70 ± 0.12	2.62 ± 0.29
spl – *cv*	7.43 ± 0.38	21.94 ± 0.76

For each pair of contrary classes the class with y^2 is given first.

(26.0 percent minus 3.2 percent) for the present crosses in which the crossover counts were made.

As expected, table 2 shows that increases in crossover values are produced by the presence of the inversions: $y^2 - w^a$ recombination increases to a value some six times its value without inversions, this factor for $w^a - spl$ is about 4, and for $spl - cv$ about 3. The differences for the values between inversion and noninversion mothers are clearly significant. The actual values reached are within the range of those customarily obtained for this region both with and without these inversions (although the effect produced here is relatively somewhat more marked in $y^2 - w^a$ compared with that in $w^a - spl$ than is the case for some other slightly different, and better controlled, crosses for this region—see REDFIELD 1955.)

Now on their face value and without further analysis these data from table 2 might tempt one to conclude that they are consistent with the hypothesis of death of eggs through some such process as autosomal nondisjunction, and that false increase of crossover values might here be produced by elimination of noncrossovers in the dead eggs. That this conclusion is not justified in this experiment is easily demonstrated by exposing a complicating cause of death. A definite clue is supplied by the facts that for mothers without inversions the hatch in the present cross was some 10 percent greater than from comparable mothers of table 1, whereas for inversion mothers it was some five percent less. The first fact shows that the intrinsic genetic viability of the eggs from noninversion mothers is markedly higher in the present cross than in the relatively more pure p-26 strain, and the second fact shows that addition of the inversions nevertheless reacts in some way to give less viable eggs than it did in the previous experiment. A concrete reason for this reversal emerges on examination of the contrary classes, which exhibit a deficiency of crossveinless adults for inversion mothers, but not for noninversion mothers. The ratio of the total of all *cv* adults to the total of all non-*cv* adults is for females without inversions 0.99; for *Cy*/+; *Payne*/+ females it is only 0.72. And for all major crossover types considered separately the same general relationship holds as a glance at the actual numbers in table 2 shows. Thus although *cv*/*cv* and *cv*/+ classes are practically equal for noninversion mothers, among the offspring of inversion mothers a large proportion of *cv* homozygotes die, and it is obvious that they die in both crossover and noncrossover classes. Whether this semilethal effect is due to the *cv* gene itself or to some mutant closely associated with that gene is not known, but it is immaterial. The effect seems not to be dependent upon a direct combination of homozygous crossveinless with any permutation of the inversion chromosomes; the classes +, *Cy*, *Dfd*, and *Cy Dfd* (not recorded) were apparently equal. If verified this suggests the additional involvement of some maternal influence on *cv*/*cv* eggs of the inversion chromosomes, but this possibility was not dealt with further—it does however fit in well with the high egg mortality, since maternally inherited effects are very apt to appear early in development.

It is interesting that the peculiar specificity of the relationship here is somewhat similar to that shown by a case recently reported by STURTEVANT (1956), in which a dominant in III causes the death of all prune (X chromosomal) flies which carry it; this latter situation involves no maternal effect.

That the semilethality of the crossveinless homozygotes is operative primarily at

the egg stage seems fairly certain, not only because there is an observed high death rate of eggs, but also because the ordinary appearance of the inversion culture bottles gave no gross indication of increased numbers of dead larvae or pupae. In view of the results it is regretted that no counts were made of pupae, or of the exact number of eggs from which the counted adults came. Such egg counts are available, however, for the next experiment reported below.

One can easily estimate what proportion of crossveinless homozygotes have died as eggs among the offspring of inversion mothers shown in table 2. Assuming the same ratio of *cv/cv* to *cv/+* adults expected for the two types of mother (that is 0.99), then the expected number of *cv/cv* adults from *Cy/+; Payne/+* mothers is 1695. The loss of *cv/cv* adults is then 469, or 14 percent of the expected total adults. This results (assuming larval and pupal death completely negligible in the crossveinless effect, which may be too extreme) in approximately 17 percent as the total expected death of eggs, which in turn gives about 14 percent for the expected value for "dominant lethals"—that is, expected if the secondary semilethal effect of crossveinless were not operating. This estimated value is not far from the ten percent demonstrated "dominant lethals" of table 1 (especially if one considers that the two separate experiments are not strictly comparable), and it would necessarily more closely approach ten percent if one could take into account whatever larval and pupal death may actually be involved in the crossveinless effect. That the larval and pupal component is, however, very small (or is even nonexistent) is indicated by the close agreement of this value of 14 percent as it stands with that obtained in the clearly more comparable experiment of table 3 which gives a value of 12 to 16 percent.

The importance of limiting the egg mortality in such studies to that irreducible minimum which is a necessary concomitant of the presence of the inversions in the mothers becomes obvious. An even simpler experiment than that just described was therefore next carried out and is reported in table 3. Mothers were used which were heterozygous for yellow^2, apricot, and split and which were at the same time of two types containing or not containing the Curly and Payne inversions.

Females without inversions were crossed to tester males (y^2 *su-w*^{a} w^a *spl*) both with and without the inversions; inversion females were crossed only to noninversion tester males. Thus there were three crosses of y^2 w^a *spl/+* females, but at the time it was impossible to carry out all three simultaneously; one of these, namely the cross of noninversion females by inversion males, was begun some six weeks later than the other two. For this reason this cross is not as strictly comparable with the other two as these two are with each other; this is true particularly in that the mothers derived later are from a different set of cultures. In general the technique was similar to that described for the preceding experiment. However it differed in that blocks of medium containing approximately 150 eggs were removed from the egg collecting trays and were placed separately on rounds of toweling paper in small Petri dishes (diameter 2 inches)—here the eggs were first counted, and a fresh supply of yeast in the form of a thin paste was then added to the paper. After the second egg count each block of medium with its paper and larvae was put into a standard culture bottle where the adults developed. Thus the counts of adults gave the desired re-

TABLE 3

Egg counts and recombination counts from y^2 w^a spl/+++ ♀♀ × y^2 su-w^a w^a spl ♂♂. *The inversions are introduced as indicated*

Inversions			
Mothers	None	None	*Cy; Payne, Dfd*
Fathers	None	*Cy; Payne, Dfd*	None
Eggs laid	10683	10216	8106
Eggs unhatched	2.2%	5.5%	18.0%
Larval and pupal deaths	6.9%	9.6%	10.9%
Crossover type			
0	4808 + 4762	4393 + 4090	2733 + 2602
1	42 + 35	48 + 47	137 + 136
2	31 + 26	33 + 37	85 + 66
1,2	0 + 0	0 + 0	1 + 0
N	9704	8648	5760
♂ X nondisjunction	Undetermined	0.07%	0.10%
Crossover values			
$y^2 - w^a$	0.79 ± 0.09	1.10 ± 0.11	4.76 ± 0.28
$w^a - spl$	0.59 ± 0.08	0.81 ± 0.10	2.64 ± 0.21

For each pair of contrary classes the class with y^2 is given first.

combination values from a known number of surviving eggs; also they gave incidentally the combined larval and pupal mortality.

The results in table 3 from parents lacking inversions are, as expected, like those shown in table 2, with 2.2 percent egg mortality (compared with the 3.2 percent of table 2), and with recombination values for $y^2 - w^a$ and $w^a - spl$ within the ordinary range. For inversion females mated to males without inversions the computations give almost identical recombination values with those shown in table 2; the egg mortality on the other hand is definitely lower (that is 18 percent instead of 26 percent); this is expected in view of the large loss of eggs in the preceding experiment among homozygous crossveinless zygotes. "Dominant lethals" here amount to 15.8 percent of the eggs laid; this compares quite well with the estimated value (eliminating the *cv* effect) of 14 percent from the earlier data.

It is true that the third cross shown in table 3, of noninversion females by inversion males, did not give precisely the results which the first experiment led one to expect (see table 1), that is identically the same egg mortality as when both parents lack inversions. However the difference as seen in the present data amounts to only 3.3 percent (5.5 percent minus 2.2 percent). Possibly the six weeks' time elapsing between the two sections of this third experiment is somehow implicated in this small difference. The particular cultures from which the parents came were of course not the same, and the environmental conditions might have differed by some unknown minor factor. This discrepancy in fact may be taken as typical of the variation exhibited by inversion crosses, depending no doubt upon extreme sensitivity to experimental conditions.

The production from the inversion mothers of a 1,2 double crossover may be noted in passing. This was a y^2 *spl* male; it was tested, and from it a y^2 *spl* stock was

derived. It could not have been the result of contamination, since no y^2 *spl* flies were then present (or had been present) in the laboratory. Such double crossovers within this small region (from the yellow locus to that of split) have previously been found in three tested instances from comparable inversion mothers (see REDFIELD 1955); none has been found among the much larger numbers of examined offspring from mothers lacking inversions. The significance of such cases is obvious in connection with our interest in the actual mechanics of pairing and the crossing over process; the X chromosomes are definitely more free to exchange very small segments when heterozygous inversions are present in II and III. These multiple crossovers within genetically quite small distances show also that it is necessary to deal with an extremely well marked chromosome if one is to pick up all crossing over produced in it by inversions in other chromosome pairs.

The simplest of calculations will give the amount of loss required from the cross without inversions to increase the recombination on the basis of elimination to the value observed in the presence of the inversions. Thus for the region $y^2 - w^a$ the number of observed crossovers is 77. If $y^2 - w^a$ crossing over is to reach a value of 4.76 (the value observed with inversions) without disturbing the crossover class, then the expected total number of adults would be 77/.0476, or 1618. The expected loss of noncrossovers is then 9704 (the observed N) minus 1618, or 8086; this is 83 percent of the observed adults, or 76 percent of the total number (10,683) of eggs laid. This results, added to the 2.2 percent of observed egg loss, in an expected total loss of over 78 percent of all eggs laid. Such an extreme egg mortality is arithmetically necessary to account for the observed increase of the crossover value in terms of elimination of noncrossovers. If we make a similar computation for the region $w^a - spl$, we get a corresponding value of 73 percent.

A comparable treatment of those two crosses given in table 3 which are reciprocal with respect to the inversion chromosomes is in one sense a better choice for such computations since the viability relationships are more alike. Here again we shall consider only the amount of elimination which would be necessary to give the increased recombination, ignoring for the moment the secondary question of the presumed means of elimination. The result is that we still find the extremely large discrepancies between the observed egg mortality and the expected egg mortality, for the latter in this case must reach 60 to 70 percent. Thus when the regions dealt with at this left end of the X are small enough that one can be certain of detecting all crossing over, the enormous values of 60 to 80 percent for egg mortality are found necessary to explain, in terms of elimination of noncrossovers, the recombination increases which are actually produced. And such extreme egg mortality as this is not exhibited by these *Cy*/+; *Payne*/+ mothers under the experimental conditions in operation here, for the observed value for total egg mortality reaches only 18 percent.

It is desirable to indicate briefly the results which can be derived from the data when they are calculated separately according to the day of age of the mothers. Unfortunately the daily totals for an age change study of crossing over in the two small regions must of necessity be very high; the data will not be presented in detail here because it is not certain the results are significant in their entirety. However

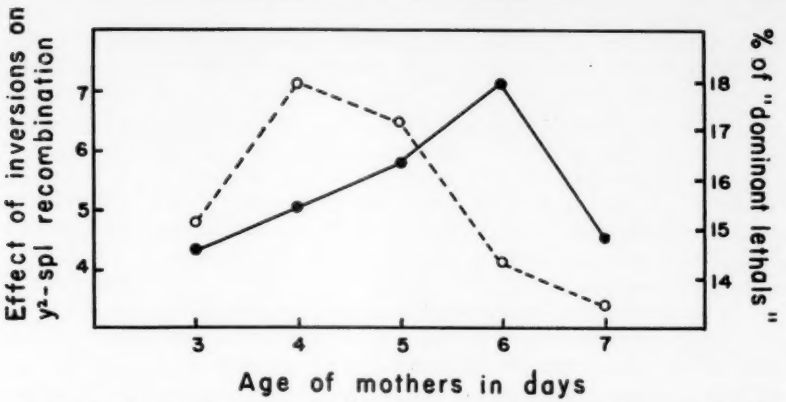

FIGURE 1.—Data the same as those for table 3, but arranged for the days taken separately. The graphs show: (1) Age change of the effect of combined Curly and Payne inversions on recombination in the region $y^2 - spl$ (solid curve—ordinates at left); this is measured by the ratio of the value for recombination from *Cy*/+; *Payne*/+ mothers, to the value from the cross without inversions. (2) Age change of the excess egg mortality given by *Cy*/+; *Payne*/+ mothers over that from mothers of the cross without inversions (broken curve—ordinates at right).

graphs of the gross results, for ages 3 to 7 days, appear in figure 1. The graph for production of "dominant lethals" (broken line) suggests a maximum of about 18 percent reached on the fourth day of life of the mothers, with a gradual decrease to about 13.5 percent on the seventh day. The graph for the recombination effect (solid line, and calculated for the sake of significance for the combined regions) shows a more gradual rise with an apparent maximum on the sixth day; and since there was no detected age change without inversions, practically all the recombination age effect as shown (expressed as the ratio of $y^2 - spl$ crossing over with and without inversions, and varying from 4.3 to 7.1) would seem to be attributable to the inversion mothers. These age change relationships should not be stressed, particularly since the possibility exists that they may be influenced by complications such as waning fertility of one sex, or of both. Still it can be stated that the maximum effect of the inversions on recombination does not, so far as the data now go, fall at the same time as the maximum for the differences for egg mortality; on the other hand the lowest values attained within this time range correspond fairly well in this respect for the two separate phenomena. This matter cannot be discussed further without additional experiments.

Effects of the Curly and Payne inversions on the entire X

In a study of the significance of the proportions of crossover types produced, it is clear that one is at an advantage if the entire X chromosome can be dealt with simultaneously. This is because the noncrossovers in a short region (such as the inclusive region from yellow to split which reaches a genetic value of less than two units in the controls of the present experiments) are in a large part undetected crossovers in other regions of the chromosome. Although this does not alter the computations

which have been given, the noncrossover strands presumably eliminated are strictly speaking among those only which are noncrossovers for the whole X. It is advisable to reach some approximation of the proportion of the emergent strands which are completely noncrossover, since the question obviously exists whether this proportion can be large enough to account for the increases in recombination by means of partial or complete elimination of these lowest rank strands. Technically it is practically impossible, of course, simultaneously to cover the complete X in as small sections as have been used here for the left end of the X. Any feasible cross will, expecially when the inversions are present, result in a value for noncrossovers which is too large by virtue of undetected double crossing over in some, if not all, regions. However we can to a degree deal with the rough estimate obtained, even though it is admittedly too large. Some years ago an experiment by REDFIELD was reported briefly which made an attempt to approach the desired complete coverage of the X chromosome (MORGAN, REDFIELD and MORGAN 1943); it was concerned with the effects of the Curly and Payne inversions on crossing over in *y² cv v f/ec car bb* females (echinus, carnation, and bobbed not previously mentioned, have the standard loci 5.5, 62.5, and 66.0 respectively—BRIDGES and BREHME 1944). Results obtained from these crosses are given in table 4; the recombination values produced are shown, also the proportions of crossover types among the observed emerging strands. These latter proportions were derived from that portion only of the total data in which all six regions could be followed at the same time. Calculations from percentages of observed strands give directly the percentages of types of tetrads (in parentheses) from which the strands arose; the equations used were obtained from WEINSTEIN'S analysis (1932, 1936). It is interesting that the proportions of observed strands from the restricted control data of table 4 agree within reasonable limits with those from data combined by WEINSTEIN (1936) from three different sources (BRIDGES and OLBRYCHT 1926; ANDERSON 1925; and WEINSTEIN) involving a total of 28,239 individuals from mothers from an entirely different combination of six X chromosome regions (*sc ec cv ct v g f*).

Table 4 shows clearly that typical recombination differences are produced by the inversions; and there is incidental evidence of the usual pattern effect along the X, namely relatively greater increases at either end of this single limbed chromosome and less marked increases in intermediate regions. The results agree, region for region, with those from experiments with less complete coverage of the X. In connection with the possibility of autosomal nondisjunction it is desirable to observe that increases are found when Curly and Payne act alone, as well as when they act in combination; the implications of this fact will be seen presently.

Of special interest are the relationships obvious from the proportions of observed emerging strands, or (if one prefers to use the data in another form) in an even more striking degree from the computed proportions of tetrads producing these strands. A simple comparison of the percentages of types of strands emerging from mothers without inversions with those from *Cy/+; Payne/+* mothers immediately makes it certain that no amount of elimination of low rank strands from the former mothers will result in the observed changed balance between the higher rank strands from the latter mothers. There seems to be no doubt that the inversion mothers have a

TABLE 4

Recombination values, percentages of crossover types of emerging strands, and computed percentages (in parentheses) of tetrads with the designated number of crossovers, from y^2 + cv v f ++/+ ec +++ car bb ♀ ♀ × In(1)HR, sc ec cv ct^6 v f car bb^1 ♂♂ *or* y^2 ec cv v f car; Y^{-bb} ♂♂

Inversions in mothers	None	*Cy*	*Payne*	*Cy; Payne*
Crossover values				
y^2 — *ec*	5.9	10.7	9.0	18.0
ec — *cv*	8.0	11.1	11.8	17.2
cv — *v*	22.2	24.7	27.3	33.8
v — *f*	20.4	22.8	29.0	35.2
f — *car*	5.9	8.0	10.6	12.6
car — *bb*	3.4	3.5	5.8	10.4
Total	65.8	80.8	93.5	127.2
N	4343	4403	3878	3349
Percent observed strands (and computed tetrads)				
Noncrossovers	42.4 (7.3)	39.0 (6.9)	33.6 (5.6)	20.3 (3.7)
Singles	45.9 (49.6)	45.6 (38.5)	45.3 (25.2)	39.5 (0.7)
Doubles	11.3 (39.2)	14.5 (47.9)	19.2 (54.7)	30.5 (43.0)
Triples	0.5 (4.0)	1.0 (5.6)	2.0 (13.4)	8.7 (35.7)
Quadruples	0 (0)	0.07 (1.1)	0.08 (1.3)	1.06 (17.0)
N	997	1539	1198	1037

The Curly and Payne inversions were present in the mothers as indicated. The data for the strands are based on F_1 males of the cross involving Y^{-bb}, for in these offspring only were all six regions simultaneously observed.

greater tendency to produce the higher rank types, and the highest ranks show the greatest difference between the two types of mother. In other words there is, in a real mechanical sense, a facilitation of exchange produced by the heterologous inversions.

A variety of manipulations of the data of this table could be carried through, but they must all on the basis of simple elimination ultimately result in a clash with observed values. If one assumes tentatively according to the computations from table 3 that about 83 percent of the adults are lost through elimination, it is evident that many more than all of the noncrossovers have disappeared, for table 4 shows only 42.4 percent of noncrossovers from noninversion mothers. It is possible to use the data of table 4 without reference to those of table 3 (in this process, of course, losing the advantage of closer though more limited marking). Thus the region from yellow to echinus which is partially comparable to the regions dealt with in the experiment of table 3, shows a recombination increase from 5.9 to 18.0; for this increase 72 percent of the adults should disappear. If the region of lowest increase is considered, namely that from crossveinless to vermilion, the necessary loss is still some 40 percent of all adults. And even this lowest value of 40 percent of adults would require an egg mortality much higher than that actually observed in the crosses where egg counts were made. Computations based upon the chromosome considered as a whole, that is in which the pattern effect is averaged, are equally futile. The crossover classes of

the noninversion cross are, as table 4 shows, 57.6 percent of the total; this amounts to 575 individuals. The total crossing over observed in the X in the presence of the combined inversions is roughly twice that shown in the absence of the inversions. Assuming this is due to simple elimination of noncrossovers, the relationships between all crossover classes remain undisturbed. The expected total with inversions is then 575/1.152, or approximately 500; this estimated total is less even than the actual number of crossovers alone. Such a *reductio ad absurdum* means, as suggested above, that there are not enough noncrossovers to account for the increase. It is necessary then to assume the disappearance also of a considerable proportion of crossover strands; these would presumably be of the next low ranking type, the single crossovers. But the addition of this further assumption proves useless also since the observed discrepancies among the remaining crossover types, namely those of higher rank, still remain unexplained on the basis of any possible elimination of lower types. Crossing over is basically different in the X chromosomes of the mothers with Curly and Payne inversions, and the difference must rest primarily upon an actual increased exchange in one pair of chromosomes, dependent upon the presence of heterozygous inversions in other chromosome pairs.

DISCUSSION

The present paper is concerned with the possibility that the interchromosomal effects of inversions on crossing over might be the direct result of selective elimination of low rank strands in those zygotes which die as eggs. Conceivably the means by which this elimination takes place might involve autosomal nondisjunction following the pairing of those non-homologous chromosomes which are members of pairs of structural heterozygotes. However on second thought one realizes that if autosomal nondisjunction were a factor of major consequence one should expect no interchromosomal effects on recombination when bivalent formation and segregation proceed normally, as is believed (see, for example, COOPER, ZIMMERING and KRIVSHENKO 1955) to be essentially the case when one chromosome pair only is structurally heterozygous. Nevertheless it is clear not only from the results provided by table 4, but from a number of other experiments also that recombination increases are produced by heterologous inversions present in just one pair of chromosomes (for examples see STURTEVANT 1919; WARD 1923; MORGAN, BRIDGES and SCHULTZ 1930, 1932, 1933; STEINBERG 1936; MORGAN, REDFIELD and MORGAN 1943; STEINBERG and FRASER 1944; and REDFIELD 1955—excepting the last, these papers are reviewed by SCHULTZ and REDFIELD 1951).

Other obvious difficulties prevent our accepting the basic implication of autosomal nondisjunction in any general explanation of interchromosomal effects of inversions on recombination. For apparently there is involved a series of uncertain events: the pairing of non-homologues of those chromosome pairs with inversions (incidentally not noticed in the salivary gland cells by SCHULTZ and HUNGERFORD); subsequent nondisjunction of the large autosomes of such pairs; and passage of noncrossovers for other chromosome pairs (of singles also?, since the noncrossovers have been shown to be too few) into zygotes which die as eggs. But such an hypothesis would still not be enough without the support of further assumptions to explain either the general pattern of increase along the chromosome limb, or the decreases

shown by particular regions. It is also not consistent with accumulating evidence of the production in the presence of heterologous inversions, of multiple crossovers of an extremely high rank and (which amounts to the same thing) of double crossovers within an extremely short region. The most striking of such classes are not found at all in controls, and they cannot be due to a shift in the proportions of already existing classes; this point has been made in a previous paper (REDFIELD 1955).

The obscure problem of the relation of primary nondisjuction of the X chromosome to recombination should be mentioned, since conceivably it might act as a factor in isolating noncrossovers to be subsequently eliminated. STURTEVANT (MORGAN and STURTEVANT 1944) states briefly that the presence of heterozygous autosomal inversions increases primary nondisjunction of the X; he regards this as a general phenomenon and attributes it to true nondisjunction rather than to chromosome elimination. Such an influence of course represents an interchromosomal effect at meiosis—it is corroborated by the data of COOPER, ZIMMERING and KRIVSHENKO (1955) for the setup which includes heterozygous inversions in the X. Of especial interest in connection with the results of the present paper is their report of one mating of females with structurally like X chromosomes and with heterozygous autosomal inversions, but this cross showed no nondisjunction of the X (N = 3,709 adults). My own data for females without the complicating X inversions have long given a somewhat consistent increase (still resulting in an extremely small value) for the detected primary nondisjunction of the X, in the presence in II and III of viable heterozygous inversions producing marked recombination increases in the X. Within a single experiment the values obtained for nondisjunction are not statistically significant, and the differences between inversion and non-inversion females are uncertain. However in my crosses females with Curly and Payne inversions are apt to give primary nondisjunction of the X in the neighborhood of 0.15 percent; this is apt to be some two or three times the value obtained in the controls without the inversions. The values shown in tables 2 and 3 agree with these results. The role of primary nondisjunction of the X in isolating noncrossovers in these inversion crosses with structurally like X's must be considered negligible. It might perhaps be thought that one could assume a practically complete masking effect due to undetected X nondisjunction through elimination in the eggs—but this, if significant in the recombination increases, should then emerge in the extreme mortality rates necessary to explain the recombination effects. And the data have been demonstrated not to exhibit such rates.

There is no doubt that females containing inverted chromosomes produce appreciable percentages of eggs which do not hatch, and that under some conditions these percentages become considerable. Now a number of quite simple minor factors exist which operate to cause the production of lethal zygotes in early stages. Ordinary semilethal relationships of a type not peculiar to females with inversions (and which may act on the egg) apparently are rather common from parents in which the inversion setup is elaborate. They may remain undetected in the absence of suitable tests, as is clear from the analysis of the second experiment of the present series. There are also other possible factors; for example unfertilized eggs are sometimes laid, but of course in *D. melanogaster* do not hatch.

But a major factor in the loss of eggs from inversion females is generally supposed

to be dependent upon four strand double crossing over within the inversion, or the two (or more) inversions together, of a given pair of chromosomes (STURTEVANT and BEADLE 1936; CARSON 1946, 1953). One would expect such a tangle to produce aberrant strands with two centromeres or with none, as the direct consequence of unravelling. This exchange would automatically eliminate a certain number of the strands deriving from the inverted pairs themselves, presumably at the egg stage. There is of course no direct evidence from recombination concerning the frequency attained by these strands in Curly-Payne females. However that single crossing over is considerable within one of the inverted sections is suggested, for example, by the data of GRAUBARD (1932) who detected inside the right inversion of Curly a frequency of surviving doubles which would indicate an expectation within this inversion of some 3.5 percent of singles—and this under conditions of structural homozygosis in all other limbs of the chromosome complement. To extrapolate from such a datum to the production in the Curly-Payne females of four strand double crossovers, perhaps mainly with one crossover in each of two limbs, and in which crossing over is increased by the simultaneous presence of inversions in four chromosome limbs involves a number of assumptions which cannot at present be made with assurance. Significantly in this connection we are interested here particularly in higher rank crossing over. Nevertheless it seems within reason to suppose that the total production of the four strand double crossovers within inversions will in Cury-Payne females reach appreciable proportions, and may very well closely approach the ten percent shown under the optimal conditions of the experiment reported in table 1 to represent the total production of "dominant lethals".

It should be clear however that the above discussion by no means excludes the possibility of the production of lethal eggs from some inversion females through autosomal nondisjunction. Indeed the data of COOPER, ZIMMERING and KRIVSHENKO (1955) for at least some inversion combinations apparently show equal numbers of male and female exceptions, and this may demonstrate that for these combinations lethal eggs are produced in considerable numbers as the result of autosomal nondisjunction. The consequences of such a situation would no doubt prove complex and far reaching.

The experiments reported in the present paper were planned as a simple test of the possibility of explaining the recombination increases for the X chromosome of Curly-Payne females as depending statistically upon the elimination in eggs of noncrossovers for the X. The real issue is whether under proper experimental conditions the increase in egg mortality produced in these inversion heterozygotes is sufficient to account for the boost in crossing over shown by the structurally like X chromosome pair. The results show that the egg mortality is much too low.

The critical third experiment involving both egg counts and counts of adults from the surviving eggs, showed that the value for the observed total egg loss in the presence of the Curly and Payne inversions was 18 percent. But the recombination increases for this experiment, those actually produced by the inversions for the yellow-split region of the X, require a total egg loss of 60 to 70 percent on the basis of elimination of noncrossovers. The situation is essentially the same when data are analyzed from adult counts only from a previous experiment in which the entire X was covered.

Simple comparison here of the proportions of crossover types demonstrates that no elimination of noncrossovers can produce the changed balance given by the inversion mothers among remaining crossover types. To put the matter in its simplest terms there are numerically not enough noncrossovers to account for the increases when all of these lowest rank strands are assumed lost. The additional disappearance of a considerable proportion of crossover strands of rank 1, the singles, would still leave unexplained the observed discrepancies in balance between the higher ranking types. And the higher the types which are compared, the greater is the difference between inversion and control mothers. There is a persistent tendency in the inversion mothers, no matter how many low ranking strands are assumed eliminated, to show in the remaining types a proportionally greater exchange between short chromosome segments. This cannot be explained as a statistical illusion depending upon simple low rank elimination; it seems to demonstrate a real facilitation of crossing over by heterologous inversions.

SUMMARY

A series of experiments is described for *Drosophila melanogaster* which tests the possibility of explaining the interchromosomal recombination effects produced by heterozygous Curly and Payne inversions, in terms of major elimination at the egg stage of low ranking strands for the X. It was found that egg mortality under proper experimental conditions is much lower than that which would be necessary to produce the observed recombination increases in the X. Simple comparison of the proportions of higher ranking crossover strands produced by the inversion females with those from control noninversion females shows further that no amount of elimination of low ranking strands can account for the changed balance exhibited among higher ranking strands. Thus these heterologous inversions so influence exchange between the two members of a structurally like pair of chromosomes that there is a much greater tendency for production of higher multiple types.

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THE FIDUCIAL LIMITS OF TETRAD-RANK FREQUENCIES*

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WEINSTEIN'S equations (1936) yield *maximum likelihood* estimates of the regional frequency distribution of crossovers in the tetrads from which a given sample of strands was derived, one per tetrad. But clearly, as with all estimates of universe parameters from sample statistics, *other* values within a certain range cannot be rejected; that range can be ascertained, for each tetrad-rank separately, from the standard error computed by a method also given by WEINSTEIN.

The standard errors of the *separate* frequencies do not, however, define the range of non-rejectable values of the rank frequencies *jointly*, where there are more than two ranks. It is with the calculation of the joint range that the present note is concerned.

Only one special case will be considered here: the frequencies of tetrads of rank 0, 1, 2, $\cdots$, k (or, further on, $k + 1$) with regard not to the regional location of the crossovers but only to the total number per tetrad, estimated from $(k + 1)$ observed strand-rank frequencies, assuming random recurrence and no sister-strand crossing over.

All possible sets of $(k + 1)$ tetrad frequencies, expressed in decimal fraction, may be thought of as lying within a k-dimensional, unit-edge hypercube, occupying a "corner" of volume $1/k!$. Somewhere within the corner is the point representing Weinstein's maximum likelihood estimate of the universe tetrad-rank frequencies for a given sample.

Now consider any other point within the corner, any other set of universe rank frequencies. Around it lie successive hyperellipsoidal shells (with increasingly flattened areas near the surfaces of the corner), each containing a finite set of points representing equally-likely sets of rank frequencies in samples from the given universe. The likelihood decreases outward from shell to shell. The sum of the likelihoods outside a given shell falls from 1 at the universe point to slightly above 0 at the plane surfaces of the corner (possibly somewhat higher at one of the surfaces).

Suppose next that we locate all points within the corner whose equal-likelihood sample shells passing through the maximum likelihood point have some particular outside likelihood sum, P. These points will themselves form a hyperellipsoidal shell, with possibly one or more flattened regions. Our problem is to locate that shell, for P generally somewhere from .01 to .05.

But the complete specifications of such a fiducial limit shell would not be very useful, because incomprehensible in any down-to-earth sense. Obviously then we have to confine our attention to particular points within the shell, to specific sets of

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possible universe frequencies: probably, by analogy with the fiducial limits of a single parameter, to some *pair* of points in opposite directions from the maximum likelihood point, crudely like the two apices of a football.

Several such pairs could be defined, at least where four or more strand ranks are observed. One pair that would seem to be of special interest is that point within the fiducial-limit shell at which the *average tetrad-rank* is *highest*, and that point at which it is *lowest*.

The conditions we have imposed are, altogether, as follows:

$$1.\ \sum_{i=0}^{k} T_i = 1 \qquad 2.\ \sum_{i=0}^{k} (s_i - \sigma_i)^2/\sigma_i = G/N \qquad 3.\ \frac{\partial}{\partial T_i}\,\bar{\imath}_T = 0$$

Here T_i represents a universe frequency of tetrads of rank i; σ_i, a universe strand frequency; s_i, an observed strand frequency; N, total strands in a sample; k, the highest observed strand rank; G, the value of χ^2 for k degrees of freedom and some specified fiducial probability, ordinarily .01 to .05.

The relation of the strand frequencies (σ's) to the tetrad frequencies (T's), assuming that there is no crossing over between sister strands but that otherwise association of strands is random, has been given by WEINSTEIN (1936) as follows:

$$\begin{aligned}
\sigma_0 &= T_0 + \tfrac{1}{2}T_1 + \tfrac{1}{4}T_2 + \tfrac{1}{8}T_3 + \tfrac{1}{16}T_4 \cdots \\
\sigma_1 &= \tfrac{1}{2}T_1 + \tfrac{2}{4}T_2 + \tfrac{3}{8}T_3 + \tfrac{4}{16}T_4 \cdots \\
\sigma_2 &= \tfrac{1}{4}T_2 + \tfrac{3}{8}T_3 + \tfrac{6}{16}T_4 \cdots \\
\sigma_3 &= \tfrac{1}{8}T_3 + \tfrac{4}{16}T_4 \cdots \\
&\cdots \\
&\cdots \\
\hline
\Sigma\sigma_i &= T_0 + T_1 + T_2 + T_3 + T_4 \cdots = \Sigma T_i = 1 \\
\bar{\imath}_\sigma &= \Sigma i\sigma_i = \tfrac{1}{2}T_1 + \tfrac{2}{2}T_2 + \tfrac{3}{2}T_3 + \tfrac{4}{2}T_4 \cdots = (\tfrac{1}{2})\Sigma iT_i = \tfrac{1}{2}\bar{\imath}_T
\end{aligned}$$

Since condition 2 has to be given in terms of s_i, and hence σ_i, the other two conditions can be imposed more conveniently by bringing them into the same terms:

$$1'.\ \sum_{i=0}^{k} \sigma_i = 1 \qquad 3'.\ \frac{\partial}{\partial T}\,\bar{\imath}_\sigma = 0$$

Condition 3′ is equivalent to 3 because $\bar{\imath}_\sigma = \bar{\imath}_T/2$ for all sets of tetrad-rank frequencies and the strand frequencies calculated from them.

We now express σ_0 and σ_1 in terms of $\bar{\imath}_\sigma$ and σ_2 to σ_k (from condition 1′ and the definition of $\bar{\imath}_\sigma$). We then derive $\bar{\imath}_\sigma$ as a function of σ_2 to σ_k by means of condition 2.

$$\sigma_0 = 1 - \bar{\imath}_\sigma + \sum_{i=2}^{k} (i-1)\sigma_i\,; \qquad \sigma_1 = \bar{\imath}_\sigma - \sum_{i=2}^{k} i\sigma_i$$

$$\frac{G}{N} + 1 - \sum_{i=2}^{k} \frac{s_i^2}{\sigma_i} = \frac{s_0^2}{1 + \sum_{i=2}^{k} (i-1)\sigma_i - \bar{\imath}_\sigma} - \frac{s_1^2}{\sum_{i=2}^{k} i\sigma_i - \bar{\imath}_\sigma}$$

Abbreviating the latter equation we have the following, in which the meanings of A, B and C will be obvious by comparison.

$$A = \frac{s_0^2}{B - \bar{\imath}_\sigma} - \frac{s_1^2}{C - \bar{\imath}_\sigma}$$

$$2\bar{\imath}_\sigma = \left[-\frac{s_0^2 - s_1^2}{A} + B + C \right] \pm \left[\left(\frac{s_0^2 + s_1^2}{A} - B + C \right)^2 - \frac{4 s_0^2 s_1^2}{A^2} \right]^{1/2}$$

Both roots satisfy the equation but if the values $A = \frac{s_0^2}{\sigma_0} + \frac{s_1^2}{\sigma_1}$, $B = \bar{\imath}_\sigma + \sigma_0$, $C = \bar{\imath}_\sigma - \sigma_1$ are substituted in the formula for $\bar{\imath}_\sigma$, it will be found that it becomes an identity on using the plus sign when $s_0^2 \sigma_1^2 - s_1^2 \sigma_0^2 \geqslant 0$, and the minus sign when $s_0^2 \sigma_1^2 - s_1^2 \sigma_0^2 < 0$.

$$2\frac{\partial \bar{\imath}_\sigma}{\partial T_i} = \left[\frac{s_0^2 - s_1^2}{A^2} \frac{\partial A}{\partial T_i} + \frac{\partial B}{\partial T_i} + \frac{\partial C}{\partial T_i} \right] - \left[\left(\frac{s_0^2 + s_1^2}{A} - B + C \right)^2 - \frac{4 s_0^2 s_1^2}{A^2} \right]^{-1/2}$$
$$\cdot \left\{ \frac{1}{A^2} \left[\frac{(s_0^2 - s_1^2)^2}{A} - (B - C)(s_0^2 + s_1^2) \right] \frac{\partial A}{\partial T_i} \right.$$
$$\left. + \left[\frac{s_0^2 + s_1^2}{A} - B + C \right] \left[\frac{\partial B}{\partial T_i} - \frac{\partial C}{\partial T_i} \right] \right\}$$

Setting this expression equal to 0, for $\bar{\imath}_\sigma$ maximal or minimal, and resubstituting for A, B and C in terms of σ_0, σ_1 and $\bar{\imath}_\sigma$ gives, after some simplification:

$$\frac{\partial A}{\partial T_i} + \left(\frac{s_0}{\sigma_0} \right)^2 \frac{\partial B}{\partial T_i} - \frac{s_1^2}{\sigma_1} \frac{\partial C}{\partial T_i} = 0 \qquad \cdots\cdot(1)$$

The partial derivatives of A, B and C with respect to T_i can be obtained on substituting their values in terms of σ_2 to σ_k. Thus

$$\frac{\partial A}{\partial T_i} = \sum_{j=2}^{k} \left(\frac{s_j}{\sigma_j} \right)^2 \frac{\partial \sigma_j}{\partial T_i}, \qquad \frac{\partial B}{\partial T_i} = \sum_{j=2}^{k} (j - 1) \frac{\partial \sigma_j}{\partial T_i} \qquad \text{and} \qquad \frac{\partial C}{\partial T_i} = \sum_{j=2}^{k} j \frac{\partial \sigma_j}{\partial T_i}.$$

It may be seen from Weinstein's equations that elimination of σ_0 and σ_1 automatically eliminates T_0 and T_1 and that $\frac{\partial \sigma_2}{\partial T_2} = \frac{1}{4}$, $\frac{\partial \sigma_2}{\partial T_3} = \frac{3}{8}$, $\frac{\partial \sigma_3}{\partial T_3} = \frac{1}{8}$, etc.

From the partial derivative of equation (1) with respect to T_2 we obtain

$$\left(\frac{s_2}{\sigma_2} \right)^2 = 2 \left(\frac{s_1}{\sigma_1} \right)^2 - \left(\frac{s_0}{\sigma_0} \right)^2 \qquad \cdots\cdot(2)$$

Taking the partials with respect to T_3, and subtracting (2) multiplied by $\partial \sigma_2 / \partial T_3$, gives

$$\left(\frac{s_3}{\sigma_3} \right)^2 = 3 \left(\frac{s_1}{\sigma_1} \right)^2 - 2 \left(\frac{s_0}{\sigma_0} \right)^2 \qquad \cdots\cdot(3)$$

So we may proceed, through T_k.

Now, substituting the values of $\sigma_2 \cdots \sigma_k$ from (2), (3), etc. into conditions 1′ and 2, we obtain

$$\left(\frac{\sigma_0}{s_0}\right) \sum_{i=0}^{k} s_i(ix^2 - i + 1)^{-1/2} = 1 \qquad \cdots\cdot(4)$$

$$\left(\frac{s_0}{\sigma_0}\right) \sum_{i=0}^{k} s_i(ix^2 - i + 1)^{1/2} = \frac{G}{N} + 1 \qquad \cdots\cdot(5)$$

where x represents $s_1\sigma_0/s_0\sigma_1$.

Eliminating σ_0 between (4) and (5) gives the following, with x as the only unknown. There are two roots, in the neighborhood of 1,

$$\sum_{i=0}^{k} s_i(ix^2 - i + 1)^{-1/2} \cdot \sum_{i=0}^{k} s_i(ix^2 - i + 1)^{1/2} = \frac{G}{N} + 1 \qquad \cdots\cdot(6)$$

approximately $[1 \pm (g/NV)^{1/2}]$ where V is the variance of the *observed* strand ranks. The larger root relates to the lower fiducial limit; the smaller, to the upper. Each can be computed, to any desired degree of accuracy, by some successive approximation method like the Newton-Raphson (WHITTAKER and ROBINSON 1944). The result can be substituted into either (4) or (5) to find σ_0 which, in turn, with x gives σ_1. Then $\sigma_2 \cdots \sigma_k$ can be calculated from (2), (3), etc. Finally the two sets of values of $\sigma_0 \cdots \sigma_k$ are put into Weinstein's equations to determine the lower and upper fiducial limits of the tetrad frequencies, as defined above.

The whole process is rather tedious, especially evaluating x. A close enough approximation, for most purposes, can be gotten from the first five terms of a Taylor-expansion of (6). Using δ to represent $(x - 1)$, T for the ratio of third central moment to variance of observed strand ranks, and F for the ratio of fourth central moment to variance,

$$\delta^2\{1 - [2T + 4\bar{\imath}_s - 1]\delta + [3.75F + 3(4\bar{\imath}_s - 1)T + 6\bar{\imath}_s(2\bar{\imath}_s - 1) + 0.25\,(1 - 3V)]\delta^2\} = G/NV \qquad \cdots\cdot(7)$$

This too has to be handled by successive approximation but seems generally to require much less effort than (6).

An example of the outcome of such fiducial limit calculations is shown in table 1. It will be noticed that upper and lower limits are not equally above and below the observed. The asymmetry is not very large, in the present case, because N is very large; it may, however, be considerable in smaller experiments.

The upper fiducial limits we have obtained are not wholly satisfactory: we have rejected universes with some tetrads of rank higher than any in the observed strands. Judging from the sharp decline in frequency with rank, in the higher strand ranks, we need to worry only about tetrads of one higher rank than the highest observed strands. For these allowance can be readily made, as follows: We calculate, for the sample with which we are concerned, the value of σ_{k+1} for which the likelihood of $s_{k+1} = 0$ is some assigned value, generally the same as the fiducial probability corresponding to our chosen value of G. (This will usually violate the rule that, in a χ^2 calculation, no expected class frequency should be less than 5. If it is preferred to adhere to that rule, σ_{k+1} may be taken as $5/N$ which is equivalent to choosing a likelihood of .0067 for $s_{k+1} = 0$.) The effects of allowing $\sigma_{k+1} \neq 0$ are: i. to replace G by

TABLE 1

Fiducial limits of strand and tetrad rank-frequencies

Rank			0	1	2	3
Strand frequency						
observed			6607	7555	1913	61
lower limit, P =	.01,	equation 7	6795.6	7463.7	1820.7	56.1
lower limit,	.05,	7	6762.5	7480.5	1836.2	56.9
lower limit,	.05,	6	6762.3	7480.6	1836.2	56.9
upper limit,	.05,	7	6456.1	7619.2	1995.4	65.4
upper limit,	.01,	7	6425.4	7630.7	2012.9	67.1
Tetrad frequency						
lower limit,	.01,	7	1096.6	7981.1	6609.8	448.6
lower limit,	.05,	7	1061.3	7957.6	6662.0	455.1
lower limit,	.05,	6	1061.0	7957.5	6662.6	454.9
maximum likelihood			904	7824	6920	488
upper limit,	.05,	7	766.8	7649.5	7196.3	523.4
upper limit,	.01,	7	740.5	7612.4	7246.2	536.9

Drosophila melanogaster X chromosome data of BRIDGES in MORGAN, BRIDGES and SCHULTZ (1935).

TABLE 2

A comparison of upper fiducial limits of strand and tetrad rank-frequencies calculated in two ways: A, highest tetrad rank same as highest strand; B, highest tetrad rank one higher than highest strand

Rank	0	1	2	3	4
Strand frequency					
P = .05 A	6456.1	7619.2	1995.4	65.4	0
B	6477.5	7607.8	1981.6	65.1	3.91
P = .01 A	6425.4	7630.7	2012.9	67.1	0
B	6446.1	7619.8	1999.2	66.2	4.61
Tetrad frequency					
P = .05 A	766.8	7649.5	7196.3	523.4	0
B	790.1	7648.6	7239.0	395.7	62.6
P = .01 A	740.5	7612.4	7246.2	536.9	0
B	763.8	7603.5	7312.4	382.6	73 7

Data of BRIDGES, in MORGAN, BRIDGES and SCHULTZ (1935).

the χ^2 for $(k + 1)$ degrees of freedom, rather than k; ii. to alter the right-hand side of (4), (6) and (7), respectively, to $(1 - \sigma_{k+1})$, $(G/N + 1)(1 - \sigma_{k+1})$ and $[(G/N + 1)(1 - \sigma_{k+1}) - 1]/V$. Once these changes have been made the procedure in evaluating $\sigma_0 \cdots \sigma_k$ is exactly as before. An example of the results, with the corresponding values for $\sigma_{k+1} = 0$, is shown in table 2.

SUMMARY

1. Although WEINSTEIN's equations (1936) give maximum likelihood estimates of the crossover-rank frequencies in the universe of tetrads from which a given sample of strands was derived, just as with every estimate of universe parameters from sample statistics other estimates within a certain range cannot be rejected.

2. Where four or more strand ranks are observed, the limits of the range form a closed shell of three or more dimensions containing, of course, infinite possible sets of tetrad-rank frequencies each of which could be regarded as a fiducial limit of the maximum likelihood estimate.

3. Among these infinite points three seem to be of principal interest:

(i.) that set of tetrad-rank frequencies for which (a) the χ^2 of the observed strand-rank frequencies would be some specified value, generally corresponding to P between .01 and .05, and (b) the average number of crossovers per tetrad is least;

(ii.) that set of tetrad-rank frequencies having (a) the same value of P and (b) the highest average number of crossovers per tetrad;

(iii.) like (ii) but including some tetrads one rank higher than the highest observed strand rank, in a proportion such that absence of strands of the same rank from the given sample would have some specified probability.

4. The lower and two upper fiducial limits, so defined, can be calculated by methods derived and illustrated here.

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INDUCED LOSS OF A RING AND A TELOMERIC CHROMOSOME IN DROSOPHILA MELANOGASTER

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RING chromosomes are potentially useful for studying the process of rejoining after chromosome breakage, since a certain portion of the restitutions should produce dicentric ring chromosomes or interlocked rings rather than structurally normal chromosomes. The relevance of ring chromosomes studies to problems of induced chromosome breakage in *Drosophila melanogaster* sperm has been recognized for some time (e.g., BAUER 1942; CATCHESIDE and LEA 1945; BAKER and VON HALLE 1955). However, the interpretation of many aspects of this work has been hampered by two factors: a comparable telomeric[3] chromosome was not available for comparison with the ring-X and marked Y chromosomes were not used with the result that loss of the X chromosome could not be detected directly.

The study being reported consists of a re-examination of the process of induced ring chromosome loss[4] coupled with the evidence this provides on the mechanism of the oxygen effect on chromosome breakage and rejoining. These data furnish no evidence against the hypothesis that the greater sensitivity of the ring chromosome to induce loss is caused by its ring nature. The data suggest, however, that some novel mechanism may underlie the formation of dominant lethals in the sex chromosomes of Drosophila.

MATERIAL AND METHODS

If a study is undertaken to determine how much of the induced loss of a ring as compared with a telomeric chromosome is caused by the ring nature of the former, it is desirable that the two chromosomes be at least roughly equal in length and have a related origin. For these reasons, the chromosomes used in our study were the X^c chromosome of L. V. MORGAN (1933) and a telomeric chromosome derived from a spontaneous opening out of this ring and designated by NOVITSKI (1949) as *In(1)EN*. The origin of the second arm of the *EN* inversion is not known but it does not contain either the Y^L or the Y^S fertility factors. Since NOVITSKI reports (*loc. cit.*) that the second arm is about one fourth the length of a normal X chromosome, as seen in larval ganglion cells, this chromosome is as long as or longer than X^c.

The Y chromosome used was the $sc^8 \cdot Y : bw^+$ chromosome of COOPER (1952) which

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[3] The term "telomeric" is used to denote a chromosome with ends as contrasted with a ring chromosome.

[4] The term "chromosome loss" is used in this paper in a broad sense to describe the processes that produce certain of the observed exceptional phenotypes and lethal zygotes. The term is not meant to imply specific mechanisms for these processes unless so stated.

carries markers y^+ from the X and an insertion of bw^+ from chromosome 2. Since the results to be reported indicate that these markers are very closely linked to the Y^S and Y^L fertility factors, respectively, loss of either one of these regions of the Y chromosome may be distinguished from a complete loss of the Y which, in itself, cannot be discriminated from loss of the X chromosome. Thus more known losses of the Y can be detected than would be possible if an unmarked or a singly marked Y were used.

The experiments were initially designed not only to obtain information on loss of the ring chromosome by radiation but also to provide evidence on the structure of the $sc^S \cdot Y:bw^+$. Males of the genotype X^c, $y/sc^S \cdot Y:bw^+$; *bw* were irradiated with doses ranging from 400 to 1800r and crossed to attached-X females of the constitution $\underline{y\ v\ bb}/Y^{cL}$; *bw*. Such a cross allows Y fragments to be recovered in F_1 attached-X females, which will be virgin since the F_1 males have only the Y^L fertility factors. (It is necessary to provide some portion of the Y chromosome to the F_1 males if sex ratio is to be determined since $X^c/0$ males are usually not viable.) F_1 females whose Y chromosome had lost either markers, or both, y^+ and bw^+, were appropriately crossed to determine if the Y^S or Y^L fertility factors were present. The F_1 females were not checked to learn whether bb^+ was present on the Y unless y^+ or bw^+ had been lost.

Although these experiments provided evidence on the structure of the $sc^S \cdot Y:bw^+$, they did not furnish reliable information, as will be explained later, on loss of the ring chromosome. Therefore, loss of the ring was studied in crosses not involving attached-X females. X^c, $y/sc^S \cdot Y:bw^+$; *bw* or *In(1)EN*, $y/sc^S \cdot Y:bw^+$; *bw* males were irradiated two to four days after eclosion and immediately pair-mated with *y v; bw* females. Twenty-four hours after mating the male was removed and mated to another *y v; bw* female for 24 hours. Each female was allowed to deposit eggs for six days and was then removed from the culture vial. This procedure gave a sample of at least two batches of sperm that were mature at the time of irradiation (see BAKER and VON HALLE 1953). The offspring from such a cross fall into the following phenotypic categories: regular *v* males and *y-bw* females; *y-v-bw* males, which arise from loss of either the ring or the Y chromosome; *y-v* males caused by loss of y^+ on the Y but not bw^+; *v-bw* males in which the bw^+ but not the y^+ marker has been lost; and *y-bw* gynandromorphs.

A similar mating procedure was used to determine the number of dominant lethals induced in the ring-bearing and telomeric-bearing males of the previously indicated genotypes. Two- to four-day-old males were irradiated and at once pair-mated with Oregon-R females. After a 24-hour mating period, the male was removed and the female placed on fresh egg-laying medium for two consecutive 24-hour periods. The male that was removed was immediately mated to another Oregon-R female for 24 hours. At the end of this period the male was removed and this female placed on egg-laying medium for two 24-hour periods. After a female was removed from an egg-laying vial, the total number of eggs laid was counted and 24 hours later a count was made of both hatched and unhatched eggs.

All irradiations were performed with a Maxitron 250-kvp X-ray machine that delivered approximately 265r/minute, at the target distance of 49 cm, with 2 mm of

aluminum external filtration, and a current of 30 ma. Dosage measurements were made with a 100-r Victoreen thimble chamber that had been calibrated in a Co^{60} gamma source whose dosage had previously been determined with a thimble chamber calibrated by the United States Bureau of Standards.

The males were exposed in lucite chambers through which either air or N_2 was passed at a temperature of 25.0 ± 0.5°C. The gas was allowed to pass over the flies for ten minutes prior to irradiation and continuously during exposure. For each experiment at a given dose level, three groups of flies were used. Two groups were irradiated simultaneously, one in air and one in N_2, and the third group was either not treated (a control in air) or was exposed to N_2 with no radiation for the length of time that the experimental series remained in this gas (a control of N_2).

RESULTS

Initial experiments

As previously noted, the initial experiments were designed to obtain evidence of the structure of the *sc*^{8}·Y:*bw*$^{+}$ chromosome as well as data on the loss of the ring chromosome. The evidence relative to the structure of this Y chromosome has been described (BAKER 1955). It is sufficient to state that a test of 94 *sc*^{8}·Y:*bw*$^{+}$ chromosomes in which one or more of the five markers (*y*$^{+}$, *bw*$^{+}$, Y^{L}, Y^{S}, and *bb*$^{+}$) were lost indicates that the most probable order of these markers is Y^{L} *bw*$^{+}$ *sfa* *bb*$^{+}$ Y^{S} *y*$^{+}$. The relative distances given in the map of this chromosome shown in figure 1 were determined by calculating the minimum number of breaks in each region that would be necessary to give the observed Y fragments in accordance with their frequency. The breakage frequencies indicate tight linkage between Y^{L} and *bw*$^{+}$, between Y^{S} and *y*$^{+}$, and between the spindle fiber attachment and *bb*$^{+}$. It is interesting to note the high frequency of breakage on either side of the centromere and the paucity of breaks distal to the fertility factors in each arm. However, this may be an artifact since the frequent fragments observed carrying just *bb*$^{+}$ can come from X as well as Y chromosome deletions.

Although extensive experiments (42,370 F_1 flies were counted) covering seven dosages between 450 and 1800r were conducted by crossing irradiated X^{c}, *y*/*sc*^{8}·Y:*bw*$^{+}$; *bw* males to *y v bb*/Y^{cL}; *bw* females, consistent sex ratios were not obtained. Loss of the irradiated ring chromosome or the entire Y would produce females with the phenotype *y-v-bw-bb*. Since the former event would convert an otherwise usually inviable 3X:2A zygote into a viable female, one would expect the female frequency to rise with increasing dosage. This result was observed but not in a consistent

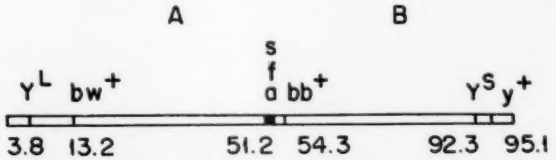

FIGURE 1.—Breakage map of the *sc*^{8}·Y:*bw*$^{+}$ chromosome assuming 100 units of length. The lengths of regions A and B are maximum estimates.

fashion. An analysis of the results indicated that the cause of the inconsistent sex ratio was either that some of the parental females had lost spontaneously the Y^{cL} chromosome or that certain of the Y^{cL} chromosomes were not able to impart viability to a male with X^c. It was found, for example, in the control experiments that a given male when crossed to the first female would produce about equal numbers of offspring of each sex; however, the cross to the second female yielded only daughters. One wonders if this ring Y is being spontaneously lost at a rather high frequency in females (MEYER and MULLER 1956 reported such an event in males). Therefore, the sex ratio in any experiment is not a true indication of ring loss but it is a function of the particular female that was used in each mating. This in no way invalidates the data on breakage of the Y chromosome although the frequency of breakage of this chromosome can be given only in terms of the total number of F_1 females rather than the total number of offspring produced.

Figure 2 shows the relation between dose and the frequency with which fragmented Y chromosomes were recovered. The Y fragments were indicated by the presence of *y-v*, *y-v-bb*, *v-bw*, or *y-v-bw* F_1 females. Although recovery of a broken Y is a rather rare event and thus the data are not as extensive as one might desire (a total of 29,222 females were scored at the various dose points), it is interesting to note that the dose curve gives no evidence of a multihit component. Whether this is fortuitious or whether breaks in this Y chromosome can heal and give terminal deletions is a question that cannot be answered satisfactorily without more experimentation.

Subsequent experiments

Since the initial experiments did not supply satisfactorily the requisite information on loss of the ring, the irradiated males were mated to *y v; bw* females. In this cross, the F_1 male viability is not complicated by the requirements of a male carrying the X^c chromosome. The first study to be made was the determination of the relative rate of dominant lethality induced in males carrying the ring and males bearing the

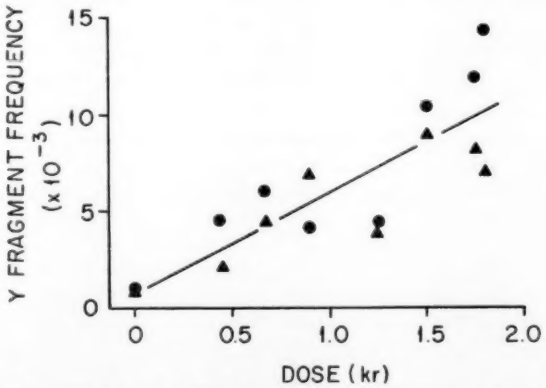

FIGURE 2.—Frequency (in terms of F_1 females) of fragments from the sc^8-Y:bw^+ recovered in F_1 attached-X females. Both sperm batches combined. Circles denote irradiations in air, triangles in N_2.

telomeric chromosome. If dominant lethality in mature sperm is a function of the chromosome breakage induced, then with equal breakage more dominant lethality might be expected in the ring than in the telomeric since torsional restitution in the former will produce a dicentric ring in the first cleavage division that might lead to death of the zygote. A semilogarithmic plot of the egg-hatch frequency as related to dose is presented in figure 3. This figure is based on the data given in table 1. Although the egg hatch frequency of the irradiated series has been corrected for the controls run simultaneously with that series, we believe that some caution should be exercised in placing too much reliance on the precise accuracy of particular points especially in the *EN* experiments, because certain of the control hatch frequencies were exceptionally low (e.g., the control experiment run with the 1000r series, first sperm batch in air, gave a hatch frequency of only 0.699). In spite of this limitation, there are still certain interesting conclusions that can be drawn validly from the data. It is obvious that more dominant lethals are induced in the ring than in the telomeric in both sperm batches for the same oxygen concentration. It is also apparent that, with a single exception, more dominant lethals were induced with irradiation in air than in

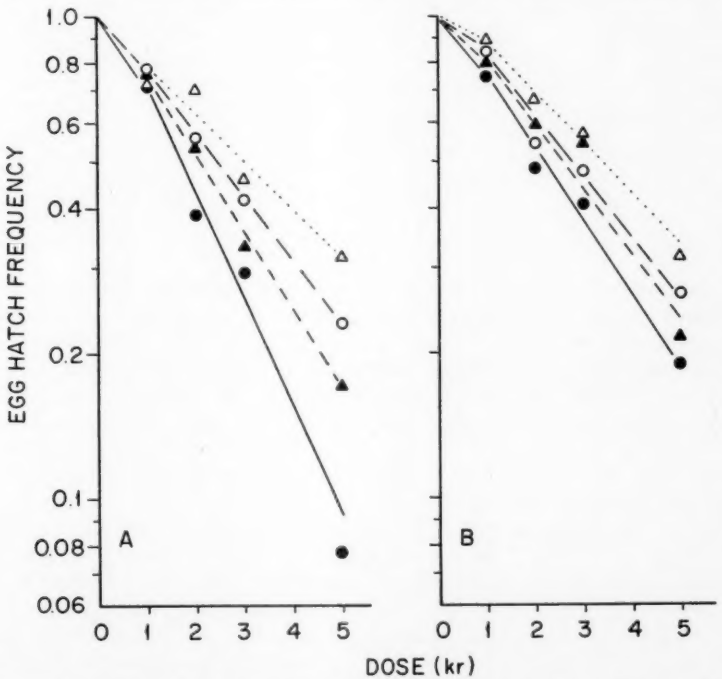

FIGURE 3.—Dominant lethal production in ring and telomeric bearing males. Circles indicate X^e irradiated males, triangles *EN* males. Closed symbols indicate exposure in air, open symbols in N_2. A indicates first sperm batch, B the second.

TABLE 1

The relation between X-ray dose, oxygen level, and the induction of dominant lethals

Dose (kr)	Gas	Sperm batch	X^e		*In(1)EN*	
			Eggs scored	Hatch freq.	Eggs scored	Hatch freq.
0	Air	1	635	0.876	595	0.699
0	Air	2	775	0.920	690	0.899
1	Air	1	813	0.716	608	0.760
1	Air	2	731	0.751	751	0.803
1	N_2	1	681	0.777	582	0.722
1	N_2	2	744	0.846	661	0.895
0	Air	1	670	0.916	747	0.898
0	Air	2	648	0.917	808	0.946
2	Air	1	801	0.390	895	0.532
2	Air	2	780	0.481	773	0.601
2	N_2	1	833	0.561	895	0.707
2	N_2	2	590	0.545	808	0.678
0	N_2	1	721	0.803	894	0.709
0	N_2	2	694	0.908	823	0.906
3	Air	1	975	0.296	659	0.334
3	Air	2	735	0.411	989	0.550
3	N_2	1	977	0.419	949	0.460
3	N_2	2	708	0.479	819	0.570
0	N_2	1	815	0.822	1476	0.894
0	N_2	2	568	0.875	937	0.962
5	Air	1	1441	0.078	1556	0.172
5	Air	2	524	0.190	981	0.218
5	N_2	1	1146	0.232	1397	0.318
5	N_2	2	473	0.268	926	0.319

N_2, irrespective of the sperm batch or X chromosome irradiated. Finally, a lowered frequency of dominant lethals is observed in the second sperm batch. This is most prominent in the air series. This result is consistent with earlier studies on dominant lethals when other chromosomes were used (BAKER and VON HALLE 1953; LÜNING 1954; TELFER and ABRAHAMSON 1954; NORDBACK and AUERBACH 1957). These results on dominant lethals suggest that the structural difference between X^e and *EN* is responsible for the higher frequency of dominant lethals induced in the former chromosome. Whether this structural difference resides in the ring nature of X^e is a point that will be discussed later.

The data on the frequency of F_1 males observed lends support to this conclusion. Table 2 is a compilation of the data on the F_1 from irradiated X^e and *EN* males. Let us consider first the total frequency of males. Two factors could contribute to the rise in the male frequency observed with increasing dose delivered to the X^e males. In the first place, if more dominant lethals are induced in the X than in the Y, then more males will be produced. Secondly, if loss of the X^e chromosome in some cases converts a female zygote into an X0 male, the male frequency will be similarly increased. The results are most striking (see fig. 4). Whereas the frequency of F_1 males from irradiated X^e males increases from a control value of 0.51 to 0.63 at 3000r, there

TABLE 2

Offspring from irradiated males having a ring or a telomeric X chromosome; X^c,y/$sc^8 \cdot Y$:bw^+; bw *or* In(1)EN, y/$sc^8 \cdot Y$:bw^+; bw ♂♂ × y v; bw ♀♀

Dose (kr)	Gas	Sperm batch	Total no. flies		Frequency of males		Freq. of *y-v-bw* males ($\times 10^{-3}$)		Freq. of *y-v* males ($\times 10^{-3}$)		Freq. of *v-bw* males ($\times 10^{-3}$)	
			X^c	*EN*	X^c	*EN*	X^c	*EN*	X^c	*EN*	X^c	*EN*
0	Air	1	4473	5209	.502	.492	3.80	1.15	0	0.04	0	0
0	Air	2	3074	3545	.505	.497	9.11	1.13	0	1.13	0	1.41
1	Air	1	2967	3051	.553	.487	8.76	1.31	0	0.98	0.34	1.64
1	Air	2	2642	3174	.533	.500	15.52	1.57	1.14	0.95	0	1.26
1	N_2	1	2649	3642	.553	.481	9.82	3.02	0	2.20	0.38	0.82
1	N_2	2	2239	4002	.546	.496	12.06	1.25	0.89	1.75	0.45	1.00
0	Air	1	2222	6059	.507	.499	4.05	0.99	0	0.03	0	0.03
0	Air	2	2675	4553	.518	.499	3.36	0.44	0	0.07	0	0.02
2	Air	1	1156	4138	.588	.493	17.30	3.14	1.73	5.07	0	0.48
2	Air	2	2064	3895	.543	.495	14.53	1.80	0.97	3.59	0	2.31
2	N_2	1	991	4477	.575	.498	14.13	4.02	3.02	2.23	0	1.34
2	N_2	2	1928	4856	.560	.499	16.60	1.85	0	1.65	1.04	0.62
0	Air	1	4126	3957	.516	.493	4.85	1.01	0.48	0.76	0	0
0	Air	2	3780	3685	.516	.489	2.38	0.81	0.03	1.36	0	0.03
3	Air	1	1197	1520	.634	.478	28.40	7.89	5.85	5.26	0	0.66
3	Air	2	2260	1677	.620	.484	26.99	0.60	3.98	4.17	0	0.60
3	N_2	1	1709	1910	.609	.514	23.41	3.66	5.85	2.09	2.93	1.57
3	N_2	2	2854	2346	.597	.486	15.07	2.98	0.70	2.13	1.05	1.28

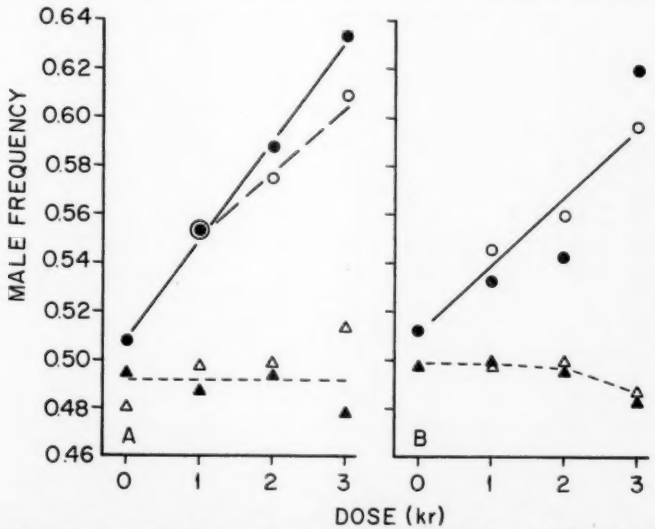

FIGURE 4.—The frequency of F_1 males from irradiated X^c males (circles) and *EN* males (triangles). Closed symbols indicate exposure in air, open symbols in N_2. A and B indicate first and second sperm batches, respectively.

is no apparent shift in male frequency when *EN* was treated. This verifies an earlier finding of C. W. EDINGTON (personal communication). The obvious implication from these data is that in *EN* males equal numbers of X and Y-bearing sperm are being eliminated by the radiation whereas in X^e males relatively more of the former sperm are being lost. These data also show the small influence of oxygen on the induction of this particular genetic effect.

As previously noted, part of the increase in male frequency could be a function of the number of X0 males observed. Now X0 males can arise in two ways, by loss of the X chromosome in a female zygote or by loss of the Y in a male zygote. The former event would cause a shift in the sex ratio whereas the latter would not. Figure 5 is a presentation of the dose relation of F_1 X0 males for both types of X chromosomes (data taken from table 2). Not only are more X0 males produced by irradiation of X^e as contrasted with *EN* males, but the spontaneous rate of their production is higher in X^e males. Since both types of irradiated males carry identical Y-bearing sperm, this difference in the frequency of X0 males must be attributed to the structure of the two X chromosomes. There is no evidence of an oxygen effect on this particular event. In addition, X0 males occur with comparable frequencies in the two sperm batches.

The final line of evidence favoring the supposition that the differences observed between X^e and *EN* is a function of the X chromosome structure is the observation that there was no difference in the frequency of Y fragments in the two cases. This can be seen in the data presented in figure 6, which relates the frequency of *y-v* and *v-bw* males (loss of only the y^+, and the loss of only the bw^+, respectively, on the sc^8-Y:bw^+ chromosome) to the dose of radiation. The data are not extensive but they

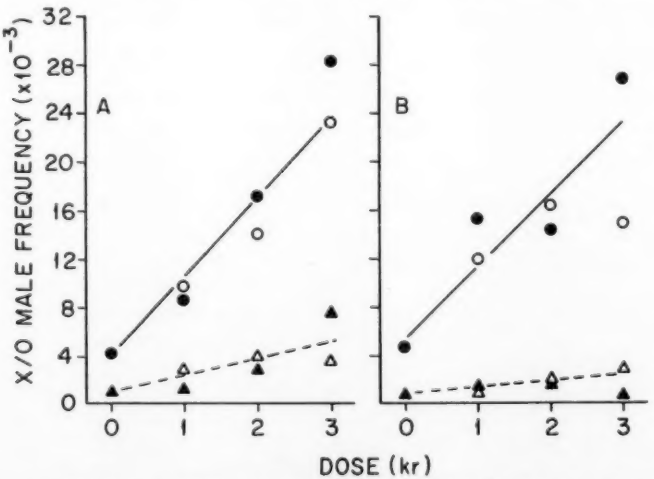

FIGURE 5.—The frequency of X0 male offspring from irradiated X^e (circles) and *EN* (triangles) males. Closed symbols indicate exposure in air, open symbols in N_2. A and B denote first and second sperm batches, respectively.

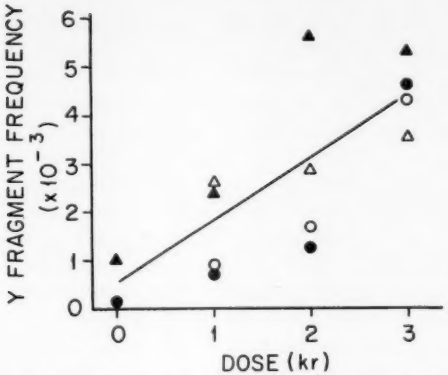

FIGURE 6.—Frequency of fragments from $sc^8 \cdot Y:bw^+$ recovered in F_1 males from irradiated X^c (circles) and *EN* (triangles) males. Closed symbols denote exposure in air, open symbols in N_2. Both sperm batches are combined.

point to the same conclusion that was reached in the initial experiments; namely, that there is no evidence of a multihit component and thus a number of these fragments may involve single breakage and healing of the Y heterochromatin.

DISCUSSION

Breakage, reunion and loss of ring chromosomes

The data gathered in this investigation provide some evidence on the events stemming from breakage of a chromosome that in turn determine the fate of the zygote receiving this chromosome. Before proceeding to relate the data to these events, let us consider the experiments in a more abstract sense. For the sake of simplicity, we shall discuss only the case where a single break is induced in a sperm. For a given dose of radiation, let the probabilities that a break will be induced in an X^c-bearing, an *EN*-bearing and a Y-bearing sperm be $P(b^c)$, $P(b^E)$, and $P(b^Y)$, respectively. Since the break can be in either the sex chromosome of the sperm or one of the autosomes, let s^c, s^E, and s^Y be the fraction of sperm of a particular type (with one break) having that break in the X^c, *EN*, or Y chromosome. Our knowledge of chromosome breakage would lead us to believe that three events can follow the production of a single break, namely, restitution, torsional restitution, and sister fusion. Let r, t, and $1-r-t$ be the probabilities that a restitution, a torsional restitution, or a sister fusion occurs, respectively.

In a ring chromosome there is an additional event that could possibly follow breakage—rotational restitution. A rotation of 360° of one broken end around the chromosome axis followed by true restitution would lead to interlocked ring chromosomes that would form a double bridge at anaphase of the subsequent mitotic division. In the following discussion the term "torsional restitution," when applied to ring chromosomes, will include the possibility of rotational restitution.

Since the production and reunion of the induced breaks has been considered, the

next question to be posed is to what measurable end effect these events lead. It is obvious that true restitution will produce no structural change in any type of chromosome, and that torsional restitution will similarly have no effect on telomeric chromosomes. However, torsional restitution in a ring chromosome will lead to an equal-armed double bridge in the first cleavage division that could produce either dominant lethality or an X0 male. Let p equal the probability that torsional restitution in X^c leads to dominant lethality and $1-p$ be the probability that it produces an X0 male. Now, sister fusion of an autosomal break would almost always lead to dominant lethality, but this is not necessarily true of the sex chromosomes. Let m, n, and o be the respective probabilities that sister fusion will cause dominant lethality if it occurs in an EN, a Y or an X^c chromosome. The corresponding probabilities that sister fusion produces X0 males are $1-m$, $1-n$, and $1-o$.

If we now turn to the data, we may draw certain conclusions about these probabilities. In the first place, it was found that there was no shift in the sex ratio when EN males were irradiated. From this, one infers that dominant lethals were induced in EN sperm with the same frequency as in Y sperm. We conclude, therefore, that EN is just as likely to have a break as Y; i.e., $P(b^E) = P(b^Y)$. Since the same autosomes are involved in the two types of irradiated males, $s^E = s^Y$. (It should be noted that with structurally normal X and Y chromosomes some investigators observe a small shift in the sex ratio. For a review of this work, see LEA 1955.) Since the data also indicate that practically no X0 males were produced when EN males were irradiated, it would be a valid approximation to let both m and n be equal to 1; i.e., sister fusion of a break in EN or in Y leads entirely to dominant lethality. This conclusion deserves some further comment. Most investigators have assumed that dominant lethality associated with chromosome breakage was caused by the genetic imbalance initiated by breakage-fusion-bridge cycles resulting from sister fusion of broken chromosome ends. However, no genetic imbalance would be caused by bridge cycles involving the Y chromosomes since males are viable with no Y or with various fragments of the Y. This finding then points to a different kind of dominant lethality associated at least with sex chromosomes in Drosophila. One wonders, for example, if the sex chromosomes are so oriented in the first nuclear cleavage spindle that any bridge formed between either of the sex chromosomes prevents the formation of daughter nuclei and thus gives rise to a lethal zygote.

Let us now determine the predictions that can be made from a model of ring chromosome loss having two additional assumptions. The first assumption is that sister fusion in the ring invariably causes dominant lethality rather than X0 males; i.e., $o = 1$. This is a reasonable guess since most of the double bridges formed at the first cleavage division would be asymmetrical—the break would have to be in the middle of the ring chromosome opposite the centromere to produce a symmetrical bridge. NOVITSKI (1955) has shown that an asymmetrical double bridge leads to breakage and inviability when present at anaphase II, and the same result might be anticipated for the first cleavage division. The second assumption is that X^c and EN are equally likely to be broken, thus $P(b) = P(b^c) = P(b^E) = P(b^Y)$ and $s = s^c = s^E = s^Y$. Although this assumption is arbitrary, it is compatible with the mitotic length of these chromosomes as seen in larval ganglion cells.

On the basis of the foregoing model, the following predictions can be made. If we let D^E and D^e be the frequency of dominant lethals induced by irradiation of EN males and X^e males respectively, then it can be shown that

(1) $D^E = P(b)(1 - r - t)$

(2) $D^e = D^E + P(b)stp/2.$

Also the frequency, F^e, of X0 males recovered from irradiated X^e males is

(3) $$F^e = \frac{P(b)st(1 - p)}{2(1 - D^e)}$$

and the frequency of all viable F_1 males recovered from an irradiated X^e male is

(4) $$M^e = \frac{1 - D^E}{2(1 - D^e)} + F^e.$$

Finally, it can be shown that from irradiated X^e males the ratio of the frequency of X0 males to the excess over $\frac{1}{2}$ of the frequency of viable males is a simple function of p.

(5) $$\frac{F^e}{M^e - 1/2} = \frac{2(1 - p)}{2 - p}$$

If the probability, p, that torsional restitution is X^e causes dominant lethality instead of an X0 male, is calculated from the data obtained on total male and X0 male frequencies, three interesting conclusions become apparent. (This calculation is tabulated in the third column of table 3.) In the first place, the values of p are all high, indicating that most torsional restitutions in the ring lead to dominant lethality. Secondly, there is no effect of oxygen concentration on this probability. Lastly, and most interestingly, it appears that torsional restitution in the second batch of sperm is less likely to lead to dominant lethality than this event in the first batch. If one assumes that at least part of the dominant lethality is produced when the dicentric bridge breaks whereas X0 males result when it does not, then this sperm-batch effect might mean that firmness of the reunited break is a function of the time interval between reunion (if part of the reunions take place in the sperm) and the formation of the bridge. The longer the interval between reunion and bridge formation, the stronger the reunion and the more resistant the bridge is to breakage.

It is obvious from equation (4) that M^e can be calculated in terms of the dominant lethals produced and F^e, the frequency of X0 males. Therefore, p can be determined not only from the observed frequency of total males and X0 males but also from the dominant lethal and X0 data. If the model is consistent with the data, these calculations should agree. In column 4 of table 3 are tabulated the values of p determined in the latter manner. These figures do not agree with the previous calculation of this parameter and display a curious anomaly; namely, the value of p appears to be dose dependent. This effect was not observed in the previous calculation and makes little sense since there is no reason for suspecting that the dosage given to a sperm could in any way influence whether a dicentric bridge in the zygote would cause the pro-

TABLE 3

Estimates of certain parameters based on a model of ring chromosome breakage and reunion

Dose (kr)	Gas	Sperm batch	$p = \frac{2M^c - 1 - 2F^c}{2M^c - 1 - F^c}$	$p \equiv \frac{D^c - D^E}{D^c - D^E + F^c(1 - D^c)}$	$M^c = \frac{1 - D^E}{2(1 - D^c)} + F^c$	M^c Observed
1	Air	1	0.924	0.892	0.538	0.553
1	Air	2	0.732	0.832	0.549	0.533
1	N_2	1	0.931	—*	—*	0.553
1	N_2	2	0.867	0.843	0.540	0.546
2	Air	1	0.912	0.963	0.696	0.588
2	Air	2	0.826	0.951	0.637	0.543
2	N_2	1	0.928	0.963	0.640	0.575
2	N_2	2	0.860	0.943	0.637	0.560

* These figures cannot be calculated since, presumably by chance, more dominant lethals were induced in *EN* than in X^c in this series.

duction of a dominant lethal or an X0 male. Thus one suspects that the anomaly resides in the data on dominant lethals. This suspicion is borne out if a comparison is made between the calculated male frequency determined by the dominant lethal and X0 male data and the actual frequency of males observed. This comparison is made in the last two columns of the table. At 2000r, the calculated and observed frequencies clearly disagree.

At first thought, it might appear that one cause of this disparity is that the model takes into account only the case where one break is found in a sperm; however, at 2000r an appreciable proportion of the sperm could have multiple breaks. In sperm with multiple breaks there should be proportionally more dominant lethals in an X^c than in an *EN* zygote because, for example, any reciprocal translocations with the ring would produce dicentrics. Proportionally more F_1 males would therefore be expected from X^c than from *EN* sperm with multiple breaks, and this is in the opposite direction from the observed effect. A possible explanation of the disagreement is based on one factor that was not measured in the experiments; namely, the frequency of postembryonic dominant lethals. Dominant lethals were measured only from egg counts and the male frequency was measured from imago numbers. Therefore, if *EN* males produced proportionally more postembryonic dominant lethals at 2000r than did the X^c males, the discrepancy could be accounted for.

One final thing should be said about the proposed model—it is not the only model of ring breakage and rejoining that will account for the results. It is possible, but in the author's opinion not probable, that very little torsional restitution takes place in a broken ring and that the radical difference between X^c and *EN* in the dominant lethal as well as the X0 male frequencies could be caused by a higher breakability of the ring chromosome. The only evidence that militates against this possibility is

that the *EN* chromosome is as long as or longer than X^c in the metaphase stage of larval ganglion cells. COOPER (1951) found that chromosomes in mature sperm, the cells irradiated in this study, are probably in a similar "metaphase" condition. Of course it may be argued that the X^c chromosome contains a region particularly sensitive to breakage that is lacking in *EN*, but there is no evidence one way or the other on this point.

The oxygen effect

These data on the loss of the ring and the telomeric chromosome also offer some evidence on the oxygen effect. It was previously noted that the data in table 3 indicate that p, the probability that a torsional restitution will produce a dominant lethal rather than an X0 male, is the same whether the males were irradiated in air or in N_2. On *a priori* grounds, it would also be reasonable to assume that s, the probability that the break will be in the sex chromosome rather than in an autosome, would be unaffected by the oxygen concentration. Therefore, the basis of the effect of decreased oxygen concentration on the induction of dominant lethals must reside either in a lowering of the value of $P(b)$ when exposures are made in N_2; or a raising of the value of r; or a lowering of the value of t in the X^c case; or, finally, a raising of the value of $r + t$ if r is greater than t.

Since there is little or no effect of oxygen tension on the induction of X0 males—the only event in our model wholly caused by torsional restitution—it would appear that: (1) the value of $P(b)$ is not affected by oxygen concentration. A lowering of the break frequency would decrease the occurrence of torsional restitution since such an event requires the chromosome to be broken. (2) The value of t is increased by lowered oxygen tension. If F^c and $1-p$ are unaffected by oxygen concentration (as shown by the data) and if the reasonable assumption be made that s is also independent of oxygen, then from equation (3) it is algebraically necessary that the increase of $1-D^c$ observed in N_2 over air must be accompanied by an increase in either or both $P(b)$ and t. Since an *increased* initial break frequency in N_2 is incompatible with the dominant lethal data, the frequency of torsional restitutions must have increased in the absence of O_2. This implies that the decreased frequency of dominant lethals observed in N_2 irradiations is caused either by an increase in torsional restitution alone, or more likely by an increase in both r and t. Unfortunately, it is not possible to get estimates of the relative values of r and t from the present experiments so a decision cannot be made between these last two alternatives.

SUMMARY

A comparative study was made of the loss of a ring, X^c, and a telomeric *In(1)EN*, chromosome induced by X-irradiation of *Drosophila melanogaster* mature sperm. Three criteria of loss were investigated: dominant lethality, sex ratio, and the frequency of X0 males in the F_1. The influence of oxygen concentration and sperm batch were also studied using these criteria of effect.

The data gathered support the following conclusions: (1) For a given dose, more dominant lethals are induced in X^c than in *EN*-bearing sperm. (2) From irradiated X^c males there is a shift in the F_1 sex ratio with dose. (3) No sex ratio shift is ob-

served when *EN* males are irradiated. This indicates that as many dominant lethals are induced in the Y chromosome ($sc^8 \cdot Y : bw^+$) as in the X chromosome (*EN*). (4) The F_1 X0 males observed come almost entirely from loss of the X^e chromosome, only rarely from loss of the Y or loss of *EN*. (5) Loss of any of the three sex chromosomes studied leads predominantly to formation of a lethal zygote rather than to an X0 male. This raises the possibility that dominant lethality caused by breakage of one of the sex chromosomes is not based on genetic imbalance resulting from bridge-breakage-fusion cycles.

A model is proposed for the consequences of rejoining after breakage of a ring chromosome that is moderately successful in explaining the difference in radiation sensitivity of ring and telomeric chromosomes. This model attributes the entire difference in sensitivity to the ring nature of the X^e chromosome.

The oxygen effect observed on dominant lethals and the lack of any appreciable oxygen effect on either the sex ratio or the frequency of X0 males favors the hypothesis that oxygen is affecting the rejoining rather than the initial breakage process.

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GENERALIZED GENETIC VARIANCE AND COVARIANCE FORMULAE FOR SELF-FERTILIZED CROPS ASSUMING LINKAGE[1]

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QUALITATIVE characters can, by definition, be readily separated into phenotypic classes having a correspondence with genotypic classes. However, in assessing the continuous variability of quantitative characters, class boundaries must be arbitrary, bearing no ascertainable correspondence with genotypic classes. This obscuration of class boundaries of quantitative characters stems from small effects of single factors relative to total variation which must be ascribable either to (1) a considerable amount of non-genetic variation with few genes segregating or (2) a large or small amount of non-genetic variation with large numbers of genes segregating. The description of a quantitative character based upon these arbitrary class frequencies lacks the desired characteristic of genetic inference that enumeration enjoys in the case of qualitative traits. However, means, variances and covariances offer a familiar way of population description and are of value in making genetic inferences in the case of quantitative characters.

In quantitative characters where the number of genes is usually assumed to be of a greater magnitude than the number of chromosomes, the effect of linkage may become important in drawing genetic inferences from these variances and covariances, especially in crops where genotypic frequencies are not in equilibrium, such as in material derived from a recent cross of inbred lines. Specifically, ROBERTSON (1952) states that in the F_2 of a cross between two inbred lines, there will be a correlation between genes at adjacent loci and any analysis will tell us about properties of blocks of genes rather than of individual genes. Several authors have considered various aspects of linkage effects on quantitative characters, although consideration has been limited chiefly to random mating populations. An analysis of quantitative characters was carried out in a classic paper by FISHER (1918) who partitioned continuous variability utilizing principles of Mendelian inheritance. FISHER indicates that in a random mating population additive genetic variance and dominance variance can be defined so their contributions to correlations among relatives are the same as without linkage. WRIGHT (1921) found that the relative increase in homozygosis in successive generations of breeding is independent of dominance, the number of factors, or linkage in absence of selection and assortative mating. WRIGHT (1933) believed that only in exceptional cases should a correlation between characters in a

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natural population be attributed to linkage in lieu of a common physiological factor because alleles at different loci, even in the same chromosome, are considered to be combined practically at random in any freely interbreeding population of long standing. DEMPSTER (1949) observed that linkage can be expected to preserve parental types in the F_2, and under the assumption of a preponderance of coupling, the F_2 variation will be greater than without linkage. This increase was said to be very large when the number of segregating loci is large. COMSTOCK and ROBINSON (1948) proposed a method of estimating the average degree of dominance in terms of components of genetic variance in a population of biparental progenies. They considered coupling phase linkages would not be a source of bias in estimates of their average weighted degree of dominance. On the other hand, the net effects of genes tightly linked in repulsion were considered to be the same as for overdominance (superiority of the heterozygote over both homozygotes) in the action of independently segregating genes even though none of the linked genes are individually more than partially dominant to their alleles. ROBERTSON (1952) also noted this similarity of effects of repulsion linkages and overdominance. COMSTOCK and ROBINSON (1952) describe an extension of one of their earlier experimental designs for differentiating between the two sources of overdominance estimates. GRIFFING (1949), discussing a constant parent regression method, considered the analysis of quantitative gene action to be disturbed by linkage in the sampling procedure of obtaining the sets of parents used from all theoretically possible homozygous genotypes.

A few authors have been concerned with linkage effects in selfed crops. BATEMAN and MATHER (1951) present a series of genetic variances and covariances for self-fertilized crops assuming no linkage in terms of what they call fixable and unfixable components and briefly suggested the effects of coupling and repulsion linkages on these components. MATHER (1949) presents the expected genetic values, assuming linkage, for some variances and covariances in the earlier generations of self-fertilized crops, but he neither clearly states the conditions under which these equations are valid nor does he generalize his results. NELDER (1952), generalizing some of MATHER'S results, presents two formulae, assuming linkage, for genetic variances arising from backcrosses and selfed lines. HAYMAN and MATHER (1955), concerned with both linkage and epistasis in self-fertilized crops, indicate how the two effects may be separated by the use of second degree statistics available from the F_2 and F_3. Although linkage is not considered, HAYMAN (1953) discusses selection with various proportions of selfing and crossing assuming homozygosity to be disadvantageous.

In considering generalized formulae for genetic variances and covariances in self-fertilized crops assuming linkage, it would also be desirable to include the effect of random mating after the F_1 but prior to initiation of continuous selfing. For example, a breeder crosses pure lines in the hope of selecting superior recombinant genotypes from segregating material, anticipating that his efforts will be hampered by the presence of linkage. This hindrance to recombination imposed by linkage of multiple factors is stressed by ANDERSON (1939a, b) who emphasizes that even with small numbers of genes in each crossover segment, the possible recombinations among the gametes of the F_1 will only be a small fraction of the total recombinations. LUSH (1948) felt that selection of superior recombinants after a cross of pure lines may be

facilitated at times by allowing, or even promoting free intercrossing after practicing any selection; the argument being given that several generations of random cross-fertilization should follow the F_1 because selfing brings homozygosis so rapidly that much of the recombination that is to occur must take place prior to initiation of continuous selfing. Knowledge of the extent to which linkage can operate to limit what the breeder may accomplish relative to the actual potential of his genetic material is needed to give perspective in the long-range planning of breeding programs. Hence the primary concern of this paper will be to quantify the effect of linkage in terms of generalized genetic variance and covariance formulae for self-fertilized crops assuming linkage, including in these formulae the effect of random mating prior to initiation of continuous self-fertilization.

DERIVATION OF FORMULAE

Assumptions. A quotation from HORNER *et al.* (1955) with regard to utilization of expected variances, covariances and means is appropriate:

> "The necessary basis for inference from variances, means or any other statistics that have been or may be used in characterizing the variation in genetic populations is knowledge of the theoretical composition (expectations or expected values) of these statistics as a function of the genetic mechanism and any other forces, e.g. selection, that are involved. . . . while a considerable variety of these genetic expressions have been developed, none has complete generality. Instead their validity rests on special assumptions about the genetic situation. The primary reason is that derivations are tremendously simplified when particular assumptions are made."

In the interest of this simplification, the following assumptions will be made: (1) normal diploid behavior at meiosis; (2) no maternal or cytoplasmic effects; (3) no position effects; (4) no multiple alleles; (5) no mutation; (6) no selection; (7) no non-allelic interaction.

The first assumption limits inferences that can be drawn from the expected values to either diploids or to amphidiploids in which multivalent meiotic associations are entirely absent or are absent in meiotic divisions giving rise to fertile gametes. The fourth premise limits inference to populations where there are no multiple alleles, though in practice one can be sure of this only in populations from a recent cross of homozygous lines. It should be emphasized, however, that crossing of pure lines is of importance in breeding self-fertilized plants. With reference to the fifth assumption, mutations, at the rate generally believed to occur, will not seriously effect the utility of expectations premised on absence of mutation.

Although some natural selection probably always exists, one can at least avoid conscious selection in planned experiments. The assumption of no natural selection is equivalent to assuming equal gametic and zygotic frequencies dependent on only the genotypes of parents. It is apparent the assumption of no nonallelic interaction (defined to occur when the effects of genes at one locus depend on specific alleles present at other loci) is restrictive.

Definitions and symbolism. We shall now be concerned with a procedure for determining a series of genetic variances and covariances for self-fertilized crops under the assumptions set forth previously. The symbolism is sufficiently general to encompass either an F_2 derived from the cross of two homozygous lines, or any other popu-

lation fulfilling those assumptions. For brevity in the following, populations will be described as F_2 populations, although it is to be understood that in general less restrictive populations are being described. Much of the notation which will be employed throughout is consistent with that of HORNER (1952) and HORNER *et al.* (1955) who originally developed some of the concepts utilized herein to derive expected variances and covariances for various nonallelic interaction models assuming independent assortment.

Let $Y = c + \sum_i y_i$ be the value of a particular genotype where c is the value of the genotype that is homozygous for the less favorable allele at all loci, summation is over all s loci, and $y_i = 2u_i$, $u_i + a_i u_i$ or 0 depending on whether the genotype at the ith locus is B_iB_i, B_ib_i or b_ib_i, respectively. The a_i may take any positive or negative value. The assumption of no epistasis, i.e. additivity of locus effects means that u_i and a_iu_i remain constant regardless of the genotypes at loci other than the ith and that the value of the total genotype is the sum of all individual locus effects. The symbols u_i and a_i have the significance of d and h/d, respectively, in the notation of FISHER, *et al.* (1932) and MATHER (1949).

Consider the subdivision of plants in the F_4 generation, e.g., into one or more groups or subpopulations such that each subpopulation traces to a different individual in any preceding generation. That is to say, plants in the F_4 generation can be partitioned into one subpopulation tracing to the F_1, as many subpopulations tracing to individuals in the F_2 as there are F_2 plants, and as many subpopulations tracing to individuals in the F_3 generation as there are F_3 plants. For logical completeness, the population in the F_4 generation can also be partitioned into subpopulations, each of the latter consisting of an individual F_4 plant. The foregoing can be generalized by stating that any F_n ($n = 1, 2, 3, \cdots$) population can be considered simultaneously as

(1) An $F_{n,1}$ subpopulation tracing to the F_1 which

(2) is composed of one or more $F_{n,2}$ subpopulations each of which

(3) is composed of one or more $F_{n,3}$ subpopulations each of which $\cdots$

(k) is composed of one or more $F_{n,k}$ subpopulations, each of which $\cdots$

(n) is composed of one or more $F_{n,n}$ subpopulations, each consisting of one individual. Hence an $F_{n,k}$ (where $k \leq n$) subpopulation consists of all of those individuals in the F_n generation descended from a common ancestor in the F_k generation.

The total genetic variance among individuals in the F_n generation can be partitioned as the variance of:

$F_{n,2}$ subpopulation means (within the F_1),

$F_{n,3}$ subpopulation means within $F_{n,2}$ subpopulations $\cdots$

$F_{n,k}$ subpopulation means within $F_{n,k-1}$ subpopulations $\cdots$

$F_{n,n}$ subpopulation means within $F_{n,n-1}$ subpopulations where an $F_{n,k}$ subpopulation mean is the arithmetic mean of all individuals in an infinite sized $F_{n,k}$ subpopulation in the F_n generation.

The total genetic variance in another generation, such as the $F_{n'}$ ($n' \geq n$) generation, can similarly be partitioned. Certain covariances of these F_n and $F_{n'}$ populations can be defined. For example, the total genetic covariance of F_n plants with the

means of their descendants in the $F_{n'}$ generation can be subdivided as the covariance of:

$F_{n,2}$ and $F_{n',2}$ subpopulation means (within the F_1),

$F_{n,3}$ and $F_{n',3}$ subpopulation means within $F_{n',2}$ subpopulations $\cdots$

$F_{n,k}$ and $F_{n',k}$ subpopulation means within $F_{n',k-1}$ subpopulations $\cdots$

$F_{n,n}$ and $F_{n',n}$ subpopulation means within $F_{n',n-1}$ subpopulations.

It is desirable to symbolize these and similar expressions in a compact form since the above are cumbersome to write out in detail. Hence

$$(p; k, k-1; n, n')$$

(where $2 \leq k \leq n \leq n'$) will be defined as the covariance of $F_{n,k}$ subpopulation means with $F_{n',k}$ subpopulation means within $F_{n',k-1}$ subpopulations. There is no necessity in limiting the definition to subpopulation means within $F_{n',k-1}$ subpopulations and a more general expression is

$$(p; k, k'; n, n')$$

where $0 < k' < k$. HORNER'S (1952) analogous expression (where $k' = k - 1$) was

$$(k, k-1; n, n').$$

The additional parameter p in present notation distinguishes those covariances where linkage is not assumed from those covariances where linkage is assumed. The symbol p_{ij}, representing the fraction of nonparental or recombinant types of gametes (with reference only to two loci; the ith and jth) in inheritance will be referred to as the *recombination fraction* or *recombination value*. With independent assortment, all $p_{ij} = 1/2$ and p in $(p; k, k'; n, n')$ becomes $1/2$. Therefore, all formulae derived herein symbolized as $(1/2; k, k'; n, n')$ indicate absence of linkage.

As specific examples of the above notation, $(p; 2, 1; 2, 4)$ refers to the covariance of genetic means for $F_{4,2}$ subpopulations with genetic values of their respective F_2 ancestors, $(p; 3, 2; 4, 4)$ refers to the genetic variance of $F_{4,3}$ subpopulations within $F_{4,2}$ subpopulations and $(p; 2, 1; 2, 2)$ refers to the genetic variance among F_2 plants. The expectations of the variances and covariances that can be referred to collectively as $(1/2; k, k-1; n, n')$ are well known for the additive model with dominance. HORNER derived the expectations for $(1/2; k, k-1; n, n')$, in general, for the additive model with dominance, a multiplicative model, a complementary gene action model, a duplicate gene action model and an optimum number model attributable to WRIGHT, all assuming no linkage. When all p_{ij} are equated to one half, formulae derived herein will reduce to HORNER'S corresponding expressions (where given) for the additive model with dominance.

Of particular significance is an identity of $(p; k, k'; n, n')$. Analogous to the basic identity of hierarchal analysis of variance and covariance, we can write

$$(p; k, 1; n, n') = (p; k', 1; n, n') + (p; k, k'; n, n')$$

whence

$$(p; k, k'; n, n') = (p; k, 1; n, n') - (p; k', 1; n, n') \qquad (1)$$

which is general for any values of k and k' where $2 \leq k' \leq k \leq n \leq n'$.

Derivation of coded genetic means. Let r_i (= 0, 1, 2) and l_i (= 0, 1, 2) represent the number of favorable genes at the ith locus in an individual in the F_k generation and of an individual in an $F_{n.k}$ subpopulation, respectively. Hence the total genotype can be described by

$$R = (r_1, r_2, \cdots, r_s)$$

and

$$T = (l_1, l_2, \cdots, l_s)$$

where s is the number of loci. Assuming the additive model with dominance, the genetic value for a single locus can be given if the number of favorable alleles is known. Similarly, the value of the total genotype can be described knowing R and T.

The genetic value of an individual in an $F_{n.k}$ or $F_{n'.k}$ subpopulation will be conditioned by the genotype of the F_k ancestor. For example, the $F_{n.k}$ subpopulation mean of coded genetic effects at the ith locus is

$$\begin{aligned} x_{i;r_i,n,k} &= E(y_i \mid r_i, n, k) \\ &= \sum_{l_i=0}^{2} (f_{h.l_i} \mid r_i) y_{i.l_i} \end{aligned} \tag{2}$$

where $y_{i.l_i}$ is the coded genetic effect of the ith locus when l_i favorable genes are present, as given previously, h refers to generations of selfing between the F_k and F_n generations, $f_{h.l_i}$ is the relative frequency of an individual in an $F_{n.k}$ subpopulation having l_i favorable genes at the ith locus such that $\sum_{l_i=0}^{2} f_{h.l_i} = 1$. In words, equation (2) states that the value of the coded $F_{n.k}$ subpopulation mean at the ith locus is equal to the average or expected value of the $F_{n.k}$ subpopulation genotype at the ith locus given the genotype of its F_k ancestor.

The enumeration of the within subpopulation frequencies of genotypes at single loci follows. When the genotype of an individual in the F_k generation is homozygous at the ith locus (i.e. $r_i = 0$ or 2), then its descendants in the F_n generation must be homozygous at that locus (i.e. $l_i = 0$ or 2). However, when an individual in the F_k generation is heterozygous at the ith locus, (i.e. $r_i = 1$), there will be three possible genotypes, B_iB_i, B_ib_i and b_ib_i, in the F_n generation at that locus with the relative frequencies g_0, g_1 and g_0, respectively, where

$$g_0 = \frac{1}{2}\left(1 - \frac{1}{2^{n-k}}\right)$$

and

$$g_1 = \frac{1}{2^{n-k}}.$$

The $F_{n.k}$ and $F_{n'.k}$ subpopulation means of coded genetic effects at the ith and jth loci have been tabulated in columns 3–6 in table 1. The following illustrates the deri-

TABLE 1

$F_{n.k}$ and $F_{n'.k}$ subpopulation means for two linked loci

Genotype in		$F_{n.k}$ subpopulation Means		$F_{n'.k}$ subpopulation Means	
F_k generation	$f_{k.r_ir_j}$	$x_{i;r_i,n,k}$	$x_{j;r_j,n,k}$	$x_{i;r_i,n',k}$	$x_{j;r_j,n',k}$
22	$f_{k.\,22}$	$2u_i$	$2u_j$	$2u_i$	$2u_j$
21	$f_{k.\,21}$	$2u_i$	$u_j + a_ju_jg_1$	$2u_i$	$u_j + a_ju_jg_1'$
20	$f_{k.\,20}$	$2u_i$	0	$2u_i$	0
12	$f_{k.\,12}$	$u_i + a_iu_ig_1$*	$2u_j$	$u_i + a_iu_ig'_1$**	$2u_j$
11	$f_{k.\,11}$	$u_i + a_iu_ig_1$	$u_j + a_ju_jg_1$	$u_i + a_iu_ig_1'$	$u_j + a_ju_jg_1'$
10	$f_{k.\,10}$	$v_i + a_iu_ig_1$	0	$u_i + a_iu_ig_1'$	0
02	$f_{k.\,02}$	0	$2u_j$	0	$2u_j$
01	$f_{k.\,01}$	0	$u_j + a_ju_jg_1$	0	$u_j + a_ju_jg_1'$
00	$f_{k.\,00}$	0	0	0	0

* $g_1 = \dfrac{1}{2^{n-k}}$ ** $g_1' = \dfrac{1}{2^{n'-k}}$

vation of one of the $x_{i;r_i,n,k}$ values:

$$\begin{aligned} x_{i;1,n,k} &= \sum_{t_i=0}^{2} (f_{k.t_i} \mid 1)y_{i.t_i} \\ &= g_0(2u_i) + g_1(u_i + a_iu_i) + g_0(0) \\ &= u_i + a_iu_ig_1. \end{aligned}$$

$x_{j;r_j,n,k}$ values were computed in a similar fashion and columns 5 and 6 were derived from 3 and 4, respectively, by replacing n by n'. The relative frequencies of the coupling and repulsion genotypes have been pooled because their genetic values are identical. Hence nine rather than ten genotypes appear here and in subsequent tables listing these genotypes.

A coded $F_{n.k}$ subpopulation mean for all s loci can be written as

$$X_{R;n,k} = \sum_{i=1}^{s} x_{i;r_i,n,k}. \tag{3}$$

The coded mean effect of the ith locus in the F_n generation will be the arithmetic mean of the coded mean effects of the ith locus of all $F_{n.k}$ subpopulations making up the F_n generation, giving

$$\bar{x}_{i,n} = \sum_{r_i=0}^{2} f_{k,r_i}x_{i;r_i,n,k} \tag{4}$$

where f_{k,r_i} is the frequency of an F_k individual having r_i favorable genes at the ith locus such that $\sum_{r_i=0}^{2} f_{k,r_i} = 1$. The f_{k,r_i} are well-known [See e.g., JENNINGS (1917)] and are given in table 2.

TABLE 2

Genotypic frequencies of single loci, f_{k,r_i}

Gamete	Frequency
B_iB_i	$f_{k,2} = v_i^2 + v_i(1 - v_i)E^*$
B_ib_i	$f_{k,1} = 2v_i(1 - v_i)(1 - E)$
b_ib_i	$f_{k,0} = (1 - v_i)^2 + v_i(1 - v_i)E$

* v_i = the population frequency of B_i $\quad E = 1 - \dfrac{1}{2^{k-2}}$

Utilizing equation (4), and tables 1 and 2, the coded mean effect in the F_n generation of the ith locus can be derived as

$$\bar{x}_{i,n} = 2u_iv_i\left[1 + \frac{a_i(1 - v_i)}{2^{n-2}}\right]. \tag{5}$$

The coded mean effect of the total genotype is

$$\begin{aligned} \bar{X}_n &= EX_{R;n,k} \\ &= \sum_{i=1}^{s} \bar{x}_{i,n}. \end{aligned} \tag{6}$$

Hence the mean of the F_n generation will simply consist of s contributions similar to equation (5), one for each locus.

Derivation of (p;k,1;n,n'). The covariance of $F_{n,k}$ subpopulation means with $F_{n',k}$ subpopulation means (within the F_1) can be symbolized as

$$\begin{aligned} (p;\, k,\, 1;\, n,\, n') &= E(X_{R;n,k}X_{R;n',k}) - E(X_{R;n,k})E(X_{R;n',k}) \\ &= \sum_{R} f_{k,R}X_{R;n,k}X_{R;n',k} - \bar{X}_n\bar{X}_{n'} \end{aligned} \tag{7}$$

where $f_{k,R}$ is the frequency of an F_k genotype specified by R. The derivation of $(p;\, k,\, 1;\, n,\, n')$ is immensely simplified by first consideration of only two loci and then generalization to s loci. The contribution of the ith and jth loci to $(p;\, k,\, 1;\, n,\, n')$ will be given by (7) with $R = (r_ir_j)$, $X_{R;n,k} = X_{i,r_i,n,k} + X_{j;r_j,n,k}$, $\bar{X}_n = \bar{x}_{i,n} + \bar{x}_{j,n}$ and $f_{k,R} = f_{k,r_ir_j}$. The latter, the f_{k,r_ir_j}, represent the relative frequencies of the (r_ir_j) genotype in the F_k generation. The f_{k,r_ir_j} are to be defined with enough generality to allow an arbitrary number of generations of random mating prior to initiation of continuous self-fertilization. Generality will also be maintained with respect to the initial phase, allowing the genotypic frequencies to be valid for both coupling and repulsion linkage phases upon suitable substitution.

Consider two alleles, b_i and B_i, at the ith locus and the two alleles b_j and B_j at the jth locus, and suppose that the F_1 produces gametes B_iB_j, B_ib_j, b_iB_j and b_ib_j with the relative frequencies w_{ij}, x_{ij}, y_{ij} and z_{ij}, respectively. Further, suppose that the recombination fraction between the ith and jth loci is p_{ij} and the descendants of this F_1, beginning with what is usually known as the F_2, are to be randomly mated for $m = 0, 1, 2, \cdots$ generations at which time the resultant population is to be selfed. At the end of these m generations of random mating, JENNINGS (1917), ROBBINS

(1918), GEIRINGER (1944 and 1945), LI (1948) and BENNETT (1954) have shown the expected relative frequencies of the B_iB_j, B_ib_j, b_iB_j and b_ib_j gametes to be $q_{ij} = w_{ij} - D_o$, $r_{ij} = x_{ij} + D_o$, $s_{ij} = y_{ij} + D_o$, $t_{ij} = z_{ij} - D_o$, respectively, where $D_o = (w_{ij}z_{ij} - x_{ij}y_{ij})\,[1 - (1 - p_{ij})^m]$. Hence if there are no added generations of random mating prior to self-fertilization, $m = 0$ and $q_{ij} = w_{ij}$, etc. Now if the relative frequencies of the B_iB_j, B_ib_j, b_iB_j and b_ib_j gametes making up what will be termed here the F_2 generation are q_{ij}, r_{ij}, s_{ij}, and t_{ij}, respectively, then the genotypic frequencies in the F_k generation of self-fertilization can be shown, by slight modification of corresponding expressions attributable to JENNINGS (1917) and ROBBINS (1918) to be those in table 3. Although the term F_k generation of self-fertilization was used in order to be consistent with the literature, especially that of HORNER (1952) and HORNER *et al.* (1955), in actuality, there have only been $k - 2$ generations of self-fertilization (after formation of the F_2). It follows then that the frequencies are valid only for $k \geq 2$.

The $f_{k,r_{ij}}$, given previously in table 2, can be verified from table 3 by obtaining appropriate marginal frequencies. For example, the frequency of B_iB_i is simply the sum of the frequencies for $B_iB_iB_jB_j$, $B_iB_iB_jb_j$ and $B_iB_ib_jb_j$.

Subpopulation means in the n and n' generations have been shown, equation (3), to be sums of the form

$$X_{R;n,k} = c + \sum_i x_{in}$$

and

$$X_{R;n',k} = c + \sum_i x_{in'}.$$

Consequently, the covariance (within the F_1) of subpopulation means in the two generations is

$$\begin{aligned}(p;\, k, 1;\, n, n') &= Cov(X_{R;n,k}X_{R;n',k}) \\ &= \sum_{i=1}^{s} Cov(x_{in}x_{in'}) + \sum_{i=1}^{s}\sum_{j>i}^{s} [Cov(x_{in}x_{jn'}) + Cov(x_{jn}x_{in'})].\end{aligned}$$

Making use of tables 1 and 3 and equation (5) we obtain

$$\begin{aligned}Cov(x_{in}x_{in'}) = 4v_i(1 - v_i)u_i^2\Bigg[1 - \frac{1}{2^{k-1}} + (1 - 2v_i)a_i\left(\frac{1}{2^{n-1}} + \frac{1}{2^{n'-1}}\right) \\ + \frac{a_i^2}{2^{n+n'-k-1}}\left\{1 - \frac{4v_i(1 - v_i)}{2^{k-1}}\right\}\Bigg]\end{aligned} \tag{8}$$

and

$$\begin{aligned}&Cov(x_{in}x_{jn'}) + Cov(x_{jn}x_{in'}) \\ &= 4u_iu_j\Bigg[\frac{a_ia_j}{2^{n+n'-4}}\left\{(q_{ij}t_{ij} + r_{ij}s_{ij})(4\rho_{ij})^{k-2} - 2v_iv_j(1 - v_i)(1 - v_j)\right\} \\ &+ (q_{ij}t_{ij} - r_{ij}s_{ij})\left\{\frac{1 - \pi_{ij}^{k-1}}{1 - \pi_{ij}} + \left(\frac{1}{2^{n-1}} + \frac{1}{2^{n'-1}}\right)\Big(a_i(1 - 2v_i) + a_j(1 - 2v_j)\Big)\right\}\Bigg]\end{aligned} \tag{9}$$

TABLE 3

F_k frequencies of the genotypes resulting from selfing a population formed from B_iB_j, B_ib_j, b_iB_j *and* b_ib_j *gametes with relative frequencies* q, r, s *and* t, *respectively**

Genotype	Frequency = f_{k,r_is_j}
$B_iB_iB_jB_j$	$f_{k.\,22} = q^2 + (qt + rs + qr + qs)E + \frac{qt - rs}{2}(\pi)_{k-1} + \frac{qt + rs}{2}(\rho^{k-2} - 1)$
$B_iB_iB_jb_j$	$f_{k.\,21} = (qt + rs + 2qr)(1 - E) - (qt + rs)\rho^{k-2}$
$B_iB_ib_jb_j$	$f_{k.\,20} = r^2 + (qt + rs + qr + rt)E - \frac{qt - rs}{2}(\pi)_{k-1} + \frac{qt + rs}{2}(\rho^{k-2} - 1)$
$B_ib_iB_jB_j$	$f_{k.\,12} = (qt + rs + 2qs)(1 - E) - (qt + rs)\rho^{k-2}$
$B_ib_iB_jb_j$	$f_{k.\,11} = 2(qt + rs)\rho^{k-2}$
$B_ib_ib_jb_j$	$f_{k.\,10} = (qt + rs + 2rt)(1 - E) - (qt + rs)\rho^{k-2}$
$b_ib_iB_jB_j$	$f_{k.\,02} = s^2 + (qt + rs + qs + st)E - \frac{qt - rs}{2}(\pi)_{k-1} + \frac{qt + rs}{2}(\rho^{k-2} - 1)$
$b_ib_iB_jb_j$	$f_{k.\,01} = (qt + rs + 2st)(1 - E) - (qt + rs)\rho^{k-2}$
$b_ib_ib_jb_j$	$f_{k.\,00} = t^2 + (qt + rs + st + rt)E + \frac{qt - rs}{2}(\pi)_{k-1} + \frac{qt + rs}{2}(\rho^{k-2} - 1)$

* The ij subscripts have been omitted from q_{ij}, r_{ij}, s_{ij}, t_{ij}, π_{ij} and ρ_{ij} for simplification.

$$E = 1 - \frac{1}{2^{k-2}} \qquad \pi_{ij} = \frac{1 - 2p_{ij}}{2}$$

$$\rho_{ij} = \frac{1 - 2p_{ij} + 2p_{ij}^2}{2} \qquad (\pi_{ij})_{k-1} = \frac{\pi_{ij} - \pi_{ij}^{k-1}}{1 - \pi_{ij}} = \sum_{\epsilon=1}^{k-2} \pi_{ij}$$

Since $k \leq n \leq n'$ and the f_{k,r_is_j} of table 3 are for $k \geq 2$, the above expressions are subject to the restriction, $2 \leq k \leq n \leq n'$. Note that when there is no linkage, i.e. $p_{ij} = 1/2$, then $q_{ij}t_{ij} = r_{ij}s_{ij} = v_iv_j(1 - v_i)(1 - v_j)$ so that equation (9) becomes zero. The total value of $(p; k, 1; n, n')$ will consist of (8) summed over all loci, s terms, and of (9) summed over all pairs of loci, $s(s - 1)/2$ terms.

$$\begin{aligned}(p; k, 1; n, n') = {} & 4\sum_{i=1}^{s} v_i(1 - v_i)u_i^2\left[1 - \frac{1}{2^{k-1}} + (1 - 2v_i)a_i\left(\frac{1}{2^{n-1}} + \frac{1}{2^{n'-1}}\right)\right. \\ & \left. + \frac{a_i^2}{2^{n+n'-k-1}}\left\{1 - \frac{4v_i(1 - v_i)}{2^{k-1}}\right\}\right] \\ & + 4\sum_{i=1}^{s}\sum_{j>i}^{s} u_iu_j\left[\frac{a_ia_j}{2^{n+n'-4}}\left\{(q_{ij}t_{ij} + r_{ij}s_{ij})(4\rho_{ij})^{k-2} - 2v_iv_j(1 - v_i)(1 - v_j)\right\}\right. \\ & \left. + (q_{ij}t_{ij} - r_{ij}s_{ij})\left\{\frac{1 - \pi_{ij}^{k-1}}{1 - \pi_{ij}} + \left(\frac{1}{2^{n-1}} + \frac{1}{2^{n'-1}}\right)\left(a_i(1 - 2v_i) + a_j(1 - 2v_j)\right)\right\}\right]\end{aligned} \tag{10}$$

where $2 \leq k \leq n \leq n'$. If all $p_{ij} = 1/2$, then all joint contributions of pairs of loci vanish.

Derivation of (p; k, k'; n, n'). The derivation of $(p; k, k'; n, n')$ as given by equation (1) requires $(p; k', 1; n, n')$ where $k \geq k'$, which may be derived by substituting k'

for k in $(p; k, 1; n, n')$. Hence from equation (10) we have

$$(p; k, k'; n, n') = 4\sum_{i=1}^{s} v_i(1 - v_i)u_i^2\left[\frac{1}{2^{k'-1}} - \frac{1}{2^{k-1}} + \frac{a_i^2(2^k - 2^{k'})}{2^{n+n'-1}}\right]$$
$$+ 4\sum_{i=1}^{s}\sum_{j>i}^{s} u_iu_j\left[(q_{ij}l_{ij} - r_{ij}s_{ij})\frac{\pi_{ij}^{k'-1} - \pi_{ij}^{k-1}}{1 - \pi_{ij}}\right. \qquad (11)$$
$$\left. + \frac{a_ia_j(q_{ij}l_{ij} + r_{ij}s_{ij})}{2^{n+n'-4}}\left\{(4\rho_{ij})^{k-2} - (4\rho_{ij})^{k'-2}\right\}\right]$$

where $2 \leq k' \leq k \leq n \leq n'$. Observe that the first line of equation (11) gives the whole of $(1/2; k, k'; n, n')$, i.e., the last two lines vanish when all $p_{ij} = 0$.

DISCUSSION

The particular case of gene frequency of one half is especially important because material from the cross of pure lines is used often experimentally and is about the only type of material that will permit positive statements concerning gene frequencies. Symbolically, gene frequency of one half is

$$v_i = 1 - v_i = 1/2$$

which also implies

$$q_{ij} = l_{ij}$$
$$s_{ij} = r_{ij}.$$

For example, assuming gene frequency of one half, the coded mean of the F_n generation becomes

$$X_n = \sum_{i=1}^{s}\left[1 + \frac{a_i}{2^{n-1}}\right]u_i. \qquad (12)$$

Observe that (12) and (5) as well as subpopulation means are independent of p_{ij}, i.e. these means will be the same whether linkage is partial or absent. MATHER (1949) noted this same phenomenon. Further simplification in variance and covariance formulae follows by letting all $p_{ij} = 1/2$, $a_i = a$ and $u_i = u$, in which case $\sum_{i=1}^{s} u_i^2$ becomes su^2 and $\sum_{i=1}^{s} a_i^2u_i^2$ becomes sa^2u^2. Under these conditions (11) reduces to the formulae of HORNER (1952) and HORNER *et al.* (1955) for the additive model with dominance. Assuming gene frequency of one half and no random mating following the cross of two pure lines so that $q_{ij} = \frac{1 - p_{ij}}{2}$ and $r_{ij} = \frac{p_{ij}}{2}$ for coupling and $q_{ij} = \frac{p_{ij}}{2}$ and $r_{ij} = \frac{1 - p_{ij}}{2}$ for repulsion, it is found that some of MATHER'S (1949) equations are special cases. For example,

$$V_{F_2} = (p; 2, 1; 2, 2)$$
$$W_{F_2/F_3} = (p; 2, 1; 2, 3)$$
$$V_{\bar{F}_3} = (p; 2, 1; 3, 3)$$
$$\bar{V}_{F_3} = (p; 3, 2; 3, 3)$$

where the left hand symbols are those given by MATHER. Under the same simplifying conditions as described immediately above, $(p; k, k-1; n, n)$ for two loci is identical with NELDER's (1952) σ^2_{lFk} equation except for a misprint in the latter in the exponent of that expression corresponding to ρ_{ij}, which should be $l-1$ instead of l.

It is instructive to consider the general formula for $(p; k, k'; n, n')$when there is no random mating prior to self-fertilization as well as gene frequency of one half. Under these conditions $(p; k, 1; n, n')$ can be considered as a special case of $(p; k, k'; n, n')$ and the latter can readily be seen to be constituted of four partitions. These four partitions are made up of terms involving

(1) u_i^2,
(2) u_iu_j,
(3) $a_i^2u_i^2$,
(4) $a_ia_ju_iu_j$.

When segregation is random, i.e. all $p_{ij} = 1/2$, then partitions (1) and (3) correspond to additive genetic and dominance variances, respectively, as given by HORNER *et al.* (1955), while partitions (2) and (4) disappear. It can be shown that in attempting to estimate additive genetic variance and dominance variance from $(p; k, k'; n, n')$ variances and covariances equivalent in k and k' under the erroneous assumption of absence of linkage one will obtain (1) + (2) and (3) + (4), respectively. Hence partitions (2) and (4) may be said to represent linkage contributions to the additive genetic and dominance variances, respectively. The linkage contribution of partition (2) is a function of the balance between coupling and repulsion linkages, number of loci, closeness of the linkages, and relative values of the favorable alleles and may be positive or negative. The linkage contribution of partition (4) depends in addition upon values of a_i but is independent of initial coupling and repulsion phases and hence will always be positive if all a_i are of similar sign.

As long as we are willing to limit discussion to $(p; k, k'; n, n')$ covariances where $2 \leq k' \leq k \leq n \leq n'$ the two restrictions, gene frequency of one half and absence of random mating, are unnecessary in defining the four partitions discussed above. That is, $(p; k, k'; n, n')$ covariances involve only terms in u_i^2, u_iu_j, $a_i^2u_i^2$ and $a_ia_ju_iu_j$ either when $k' \geq 2$, or when $k' = 1$ if there is no random mating and gene frequency is one half. But when $k' = 1$ and there is random mating prior to selfing or gene frequency is not one half, other terms are encountered. If one were to attempt estimation of those terms involving u_i^2 and $a_i^2u_i^2$ from $(p; k, 1; n, n')$ covariances (where the k are equivalent) incorrectly assuming the absence of linkage, the estimates would be discovered to be confounded also with terms involving $a_iu_i^2$, $a_iu_iu_j$ and $a_ju_iu_j$ in both instances and, in addition, by two instead of the usual single series of terms in $a_ia_ju_iu_j$ in the second instance. However, not all of these additional terms will vanish when segregation is random. A definition of the additive genetic and dominance variance comprising the most general form of the $(p; k, 1; n, n')$ covariances can be obtained by partitioning individual locus effects of $F_{n.k}$ subpopulations. If additive and dominance effects of these individual loci are defined by a least squares solution of subpopulation means, then additive and dominance partitions of $(p; k, 1; n, n')$ can be obtained which, when $k = n = n'$ will reduce to the form as given by WRIGHT (1935) and COCKERHAM (1954). With this definition of terms additive genetic vari-

ance consists of terms involving u_i^2, $a_i u_i^2$ and $a_i^2 u_i^2$ and is a function of n as well as k. Dominance variance, on the other hand, although involving terms only of the form $a_i^2 u_i^2$ in common with partition (3) is of different form than (3) except when gene frequency is one half. Neither variance involves the recombination fraction p_{ij}. In addition to these components, four other types of partitions, representing covariances between loci in different generations, will appear, all of which will vanish if segregation is random and two of which will also vanish if gene frequency is one half or if there is no inbreeding.

Certain limiting values of the most general expressions of $(p;\ k,\ 1;\ n,\ n')$ and $(p;\ k,\ k';\ n,\ n')$ covariances will considerably reduce their complexity even though gene frequency is not one half. For example, if there is either no dominance at any loci (all $a_i = 0$) or if homozygosity is reached (n increases without limit) only terms involving u_i^2 and $u_i u_j$, corresponding to partitions (1) and (2), respectively, will remain.

A breeder may be interested in one or more generations of random mating prior to initiation of continuous self-fertilization in order to allow more recombination to take place before homozygosis is induced. However, even with random mating, linkage will still hinder recombination. Knowledge of this hindrance to recombination imposed by linkage is useful in making inferences from experimental data and for deductions concerning logical breeding programs. A means of measuring this hindrance to recombination is to consider the change in linkage contributions (partitions (2) and (4) above) from generation to generation of random mating. A change in absolute units is difficult to interpret but the change from generation to generation of random mating as a fraction of the original contribution is more susceptible to interpretation. A large relative change would indicate little hindrance to recombination in contrast to the greater limitation to recombination indicated by a small relative change.

GATES (1954) considered the relative rate of change of linkage contribution from generation to generation of random mating for those variances and covariances which can be symbolized as $(p;\ 2,\ 1;\ n,\ n')$. Because the linkage contribution expressed as partition (4) above becomes negligible in advanced generations only the linkage contribution of partition (2) was considered. The hindrance to recombination will be a function of the total length of chromosome in crossover units as well as the intensities of the linkages. Assuming many genes located randomly in the chromosome, it was demonstrated that the relative rate of change of the linkage contribution from generation to generation of random mating of a chromosome of 50 crossover units in length is -0.136, which is about 2/3 that of a chromosome of 100 crossover units in length. The negative value indicates that the expected rate of change of the linkage contribution will decrease every generation.

SUMMARY

Expected values of all possible genetic variances and covariances among and within populations which reproduce by self-fertilization were derived for the additive model with dominance, assuming linkage, but excluding maternal and cytoplasmic inheritance, position effects, selection, mutation, multiple alleles, nonallelic interac-

tion and nonnormal diploid behavior at meiosis. Since gene frequency of one half was not assumed in derivation of these formulae, the results are not of necessity limited to populations immediately following the cross of two homozygous lines as long as the genetic frequencies of the initial population are specified. The symbolism is also sufficiently general so as to allow the initial population to be randomly mated following the cross of isogenic lines, but prior to initiation of continuous self-fertilization.

With two further assumptions, gene frequency of one half and no random mating, all genetic variances and covariances can be seen to be made up of four partitions. Two of these partitions correspond to additive genetic variance and dominance variance formulae derived assuming independent segregation and gene frequency of one half. The two remaining partitions both represent linkage effects; one is associated with additive gene effects and the other with dominance effects. Their bearing on estimates of additive and dominance variance is discussed briefly.

A measure of the hindrance to recombination (imposed by linkage) from generation to generation of random mating for a special class of genetic variances and covariances, symbolized as $(p;\ 2,\ 1;\ n,\ n')$, was noted as an application of the formulae derived.

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THE MEIOTIC BEHAVIOR OF REVERSED COMPOUND RING X CHROMOSOMES IN DROSOPHILA MELANOGASTER[1]

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A reversed compound ring X chromosome is composed of two complete, or essentially complete, X chromosomes, each attached to the same centromere at one end (as in attached-X chromosomes) with the distal tips of the two chromosomes attached to each other by a heterochromatic segment, thus forming a continuous ring, the component chromosomes of which can synapse with each other simply by the collapsing of the ring. Such a compound ring was first synthesized by NOVITSKI (1954), but the absence of heterozygous markers in this ring greatly limited the analysis of this particular compound.

An analysis of reversed rings is of interest in a number of connections. First, even though only three of the six possible compound X chromosomes have been analyzed extensively, it is already evident that the results from all these compounds cannot be rationalized on the basis of what is known about crossing over in normal (unattached) X chromosomes. It is therefore of some importance that a study be made of the behavior of the various compound X chromosomes for the purpose of further testing the current notions about chromosome behavior. Second, in common with other ring chromosomes, reversed rings provide a test that can distinguish between crossing over taking place among all four nonsister chromatids of the tetrad with no sister-strand exchange as contrasted to crossing over occurring between only two of the four nonsister chromatids with sister-strand exchange then randomizing the strands (as suggested by LINDEGREN and LINDEGREN 1937; SCHWARTZ 1953). Third, reversed rings are structurally very similar to reversed acrocentric compound X chromosomes (a reversed acrocentric compound is structurally similar to an attached-X in which the centromere has been moved from a median position to a subterminal one), which behave extremely atypically in two respects during meiosis when contrasted to either free X or attached-X chromosomes (SANDLER 1954). Specifically, (1) in crosses of females that carry reversed acrocentric compounds, the level of exchange (as measured by the rate of homozygosis) is markedly affected by whether these compound-bearing females carry a homolog for the compound (the Y chromosome), the exchange frequency being much higher when they do. (2) The frequency of one-exchange tetrads (the term "tetrad" is used here to mean the complex of the four X's formed by replication of the two chromosome elements of the compound chromosome, and does not include the heterochromatic homolog, if any) is very nearly, or perhaps actually, zero, although there is a high frequency of

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both no-exchange and two-exchange tetrads. It is, therefore, of interest to compare the meiotic behavior of reversed acrocentrics and reversed rings. Finally, physical considerations suggest that ring chromosomes might, after crossing over or replication, be mutually interlocked so that in the progeny of ring-bearing parents there would be a reduced recovery of rings owing to their elimination as interlocked complexes. Reversed rings represent a situation in which genetic evidence for interlocking of rings might be obtained.

Results indicate the following: (1) The data from reversed rings are consistent with the usual assumptions about the nature of crossing over (i.e., that all four nonsister chromatids participate in exchange at random, with sister strands never crossing over) and are inconsistent with the assumption of sister-strand crossing over as outlined here. (2) Reversed rings and reversed acrocentrics are similar in that both have (a) a reduced frequency (or absence) of single exchanges as compared with exchanges of ranks zero and two, (b) about the same frequency of no-exchange and double-exchange tetrads, (c) about the same distribution of exchanges along the length of the chromosome, and (d) the same reduction in crossing over when the parental compound-bearing females do not carry a homolog for the compound. (3) There is a sizable reduction in the recovery of reversed rings in the progeny of compound-bearing females in excess of that which can be accounted for by either reduced viability of ring-bearing individuals or by a tetrad analysis, suggesting the possibility of unresolved interlocked complexes in reversed rings. However, reasons for believing that this reduction in the recovery of rings is caused by some factor other than interlocking of rings will be considered.

Synthesis of reversed rings

The reversed ring used in this analysis was synthesized in two steps: (1) constructing an attached-X chromosome homozygous for distally placed heterochromatin and (2) attaching these heterochromatic regions to form a ring (fig. 1).

The precise method employed involved constructing a special attached-X chromosome. One arm of this chromosome was *In(1)sc*^{8} (a long inversion with a large block of proximal heterochromatin placed distally and carrying a normal allele of *y* distal to this heterochromatin) carrying the markers *cv*, *v*, and *f*. The other arm was a crossover product of *In(1) sc*^{81} and *In(1)EN*, making the chromosome similar to the *sc*^{8} inversion distally but different proximally in that it carries an amount of heterochromatin different from *sc*^{8}, and carries a mutant allele of *y* (in addition to the normal allele of *y* carried at the tip). This arm was marked by the mutants *m* and *car*. Such attached-X females with no homolog were X-irradiated with approximately 2000r and crossed to attached-XY (YSX.YL), *y B* males carrying no homolog. The female progeny from this cross were examined for cases in which the attached-X chromosome had lost both distal *y*$^{+}$ loci simultaneously (i.e., *y* females). Such an attached-X would be recoverable, since it would carry a mutant allele of *y* proximally. Two ways in which such loss might be accompanied by the conversion of the attached-X chromosome to a reversed ring are shown schematically in figure 1.

Several possible reversed rings were obtained from this cross; one of the lines so obtained was used for analysis. A cytological examination of larval neuroblast

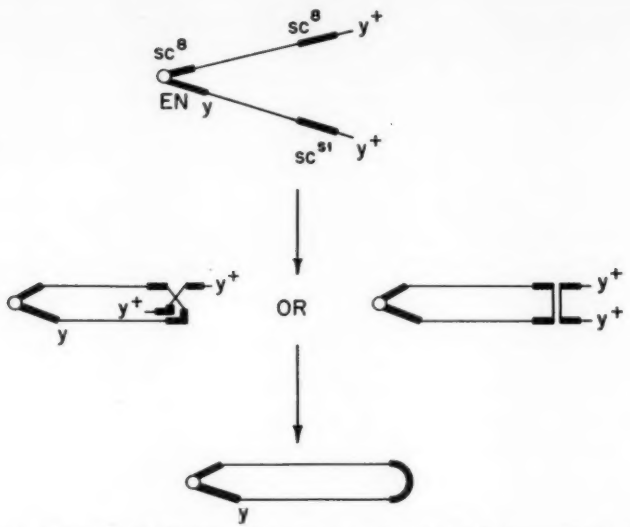

FIGURE 1.—The origin and structure of the reversed compound ring X chromosome. Line 2 shows two possible ways in which the attached-X, shown on line 1, might have been converted to the reversed compound ring, shown on line 3.

tissue from this line showed that it did carry a large ring X chromosome. Another cytological check at the end of the genetic analysis reconfirmed the presence of a ring.

METHODS AND RESULTS

The major genetic analysis performed on the reversed ring is as follows: Females carrying reversed rings, hemizygous for the mutant allele of *y* and heterozygous for the recessive markers *cv*, *v*, *m*, *f*, and *car*, which are 19.3, 3.1, 20.6, and 5.8 standard crossover units apart respectively, and carrying FR2 (a heterochromatic chromosome equivalent to the long arm of the Y chromosome and marked by the normal allele of *y*), which acts as a homolog for the compound, were crossed to *y B* males carrying the attached-XY chromosome and no homolog. It is not known for any marker whether it is in coupling or repulsion with any other marker because, although the markers originally went into the compound as *cv*, *v*, and *f* on one arm and *m* and *car* on the other, crossing over will change these relations. The progeny (F_1) from this cross were scored, and every *y* (but not *cv*, *v*, *m*, *f*, or *car*) female recovered was crossed to sibling males. In the F_2 (the progeny of these *y* females), females were scored. The coupling relations among the markers in the rings carried by the original parental females could be determined from the homozygous female progeny (of single females) in the F_1 and from the results of the progeny tests. This is so because if a female carries a reversed ring with any two markers in coupling, then a double exchange (one crossover to the right of the rightmost marker and the other to the

left of the leftmost marker) can result in a single female offspring homozygous for both markers. If, on the other hand, the original ring carried the two markers in repulsion, then, as in attached-X chromosomes, it requires an exchange of rank greater than two (such exchanges are assumed to occur sufficiently infrequently so that the error introduced by them here is small enough to be ignored) to produce an offspring simultaneously homozygous for both markers.

The progeny from the original cross are given, according to the determined genotype of the parental females, in table 1. The last five lines in the table are a summary. Since male progeny (*B* or *y B*) are recovered in this cross only when eggs are fertilized by sperm carrying the YSX.YL chromosome, and since female progeny are recovered only if eggs are fertilized by nullo-XY sperm, it is necessary, for male and female classes to be directly comparable, to correct the male classes for meiotic loss of the YSX.YL chromosome (SANDLER and BRAVER 1954). Experience has shown that the best correction is made by increasing the male classes by 16 percent. The corrected figures for the male classes appear in the first two lines of the summary. Although these corrected figures will be used throughout the analysis, it should be noted that none of the general conclusions reached depend on this correction. The numbers given in the lines of table 1 designated as "Matroclinous females" and "Total homozygous females" are obtained by deciding from the genotype of the parental female and from the observed homozygotes, how many of the *y* females scored were homozygous for wild type alleles, and assigning those to the homozygous class.

These data have been treated in a number of ways, which will be considered in the following sections.

ANALYSIS OF RESULTS

On the frequency of exchanges of rank one

A direct determination of the frequencies of the different rank exchanges in the reversed ring is not immediately possible because, for an estimate of these frequencies, the relative viability of ring-bearing females and males must be known. It is, however, still possible to determine whether single exchanges are absent, or at least very reduced in frequency, in the following way. A reversed ring compound, heterozygous for the markers *cv*, *v*, *m*, *f*, and *car*, the coupling relations among which may be symbolized *cv v f/m car*, for example, can be converted to one of the constitution *cv v car/ m f* by certain single exchanges or by certain double exchanges. A ring in which the coupling relations have been changed by crossing over will be referred to as a transpose. Now, since homozygous rings come only from double exchanges (exchanges of rank greater than two will not be considered; the conclusions to be drawn will not be affected by this exclusion), whereas a transpose may come from either a single or a double exchange, it is possible to determine, from the observed number of homozygotes, the number of transposed rings that are accounted for by double exchanges; the remainder presumably being caused by singles.

In practice, a transposed ring is not phenotypically different from the original ring, but, owing to the change in coupling relations, the difference can manifest itself in progeny tests. The data from the progeny tests have therefore been treated as follows. Parental females were chosen whose homozygous F_1 progeny indicated

TABLE 1

Results from matings of females carrying reversed compound ring X chromosomes and FR2 to males carrying the attached-XY chromosome marked with y B *and carrying no homolog. (Classes marked by an asterisk have been corrected for meiotic loss.)*

Phenotype of progeny	Genotype of parental ring-bearing females																
	cv v f / m car	cv v car / m f	cv m car / v f	cv m f / v car	cv v m / f car +	cv v f / car m	cv m f / car v	cv m / v f car	cv v / m f car	v m car / cv f	v m f / cv car	cv v m f / car	cv f car / v m	cv v m / car f	v m f car / cv	cv v m / f car	Total
B♂♂	960	676	834	603	636	1,486	909	979	732	37	620	936	246	218	101	140	
y B ♂♂	95	66	56	76	60	147	91	86	74	5	56	88	23	29	9	13	
y ♀♀	294	211	245	228	214	536	325	314	267	10	232	342	89	73	29	49	
+ ♀♀	1	0	1	0	0	1	1	2	1	1	0	1	0	0	0	0	
y cv ♀♀	2	2	2		2	3	3	3			9	4	3	1	3	1	
y v ♀♀	18	16	15	22		22	46	23	23		3	2		1			
y m ♀♀	41	21	17	11		66	6	33	12			2	1				
y f ♀♀	8	3	1	1	1	6	2	1	1	1	2	10	1	7		4	
y car ♀♀	2	2	7	3	1	2	2	1			4	18		1			
y cv v ♀♀	19	8				5			8								
y v f ♀♀	17		19			12		13									
y m car ♀♀	7		7														
y v car ♀♀		6		9					16			3			1		
y m f ♀♀		19		11	2		8	17									
y cv m ♀♀			9	3			9				11	13	6	5		3	
y v m ♀♀					7			5	3				1			1	
y f car ♀♀					1	6	3			1			3				
y cv f ♀♀																	
y cv v f ♀♀	10					5											

y cv v car ♀♀		4															
y cv m car ♀♀			3														
y cv m f ♀♀				4			1										
y cv v m ♀♀					4							6		4		1	
y v m f ♀♀					1						17	11					
y m f car ♀♀							8		5								
y v f car ♀♀						15		14									
y v m car ♀♀														3			
y cv v m f ♀♀					3							9					
y v m f car ♀♀					2										1		
y cv v f car ♀♀						5											
y cv m f car ♀♀							1										
y cv v m car ♀♀														1			
y cv v m f car ♀♀					1												
B ♂♂*	1,142	804	992	718	757	1,768	1,082	1,165	871	44	738	1,114	293	259	120	167	12,034
y B ♂♂*	113	79	67	90	71	175	108	102	88	6	67	105	27	35	11	15	1,159
Matroclinous ♀♀	292	209	243	228	189	521	325	304	263	9	194	300	86	64	24	48	3,299
Total homozygous ♀♀	126	83	82	64	50	162	89	120	72	3	84	120	18	32	10	11	1,126
Exceptional ♀♀	1	0	1	0	0	1	1	2	1	1	0	1	0	0	0	0	9

that they were of the constitution *m f/v car* or *m car/v f* (*cv* was ignored because homozygosis for this marker is rare and its coupling relations with the other markers is consequently difficult to determine). It should be noted that the females used in this test comprise only a small fraction of the females whose progeny are given under these same genotypes in table 1. This is because the genotypic determinations in table 1 involve both the F_1 and F_2, whereas the determinations in this test are made only on the basis of the F_1. The nonhomozygous female progeny from parental females of these particular constitutions were classified, from the results of the progeny tests, according to whether they were transposed, nontransposed, or whether this was indeterminate. This last category comes from cases in which the critical homozygous types failed to appear among the F_2.

Suppose, for instance, that the female progeny of an original parental female included ten *y* females, one *m car* female, one *v f* female, two *v* females, and one *f* female. From the *m car* and *v f* offspring, the maternal genotype can be specified as *m car/v f*. The ten *y* females are then mated, and their female progeny scored. If, among the progeny of any such *y* female, a *m car* or a *v f* homozygote appears, the *y* female is classified as a nontranspose; if either a *m f* or a *v car* homozygote is recovered, the *y* female is classified as a transpose; if none of these types appear, a classification cannot be made. We may note two points about this analysis. First, the selection of the parental females is based solely on homozygotes for two markers simultaneously (*m car* and *v f* in the example); there is therefore no selection with respect to homozygotes for markers appearing singly, and these are the homozygotes that will be used in the comparison to follow. Second, the homozygous products of the very same exchanges determine whether a particular ring is a transpose; that is to say, the relative probabilities of classifying a ring as either a nontranspose or a transpose ought to reflect the frequencies of these types in the sample.

There is one additional complication. Occasional crossovers do occur between *v* and *m* and between *f* and *car*. Double exchanges, in which one of the crossovers is of one of these types can produce offspring homozygous for single markers and transposes that may be symbolized as *v m*/++ or *f car*/++. These transposes will be referred to as *v–m* transposes. The occurrence, among the progeny of a single female, of a homozygote for *v* and *m* simultaneously (or for *f* and *car* simultaneously) indicates that the parental female was of the constitution *v m*/++ (or *f car*/++). The proportion of females of this constitution, among all females, that can be so classified directly from the homozygotes among their progeny has been estimated in these experiments simply by classifying original parental females according to whether they were of this constitution from the F_2 data only (this can be done for every female without ambiguity) and then checking on the proportion of these that could have been so classified from the F_1 results only. This classification was possible in 74 percent (48/65) of the cases of *v m*/++, and in 51 percent (21/41) of the cases of *f car*/++.

In this experiment there were 27 females whose multiple homozygous F_1 progeny showed that they were of one of the desired constitutions (*v f/m car* or *v car/m f*). The F_1 progeny from these females included, in addition to those females homozygous for more than one marker at a time, 452 *y* females and 103 females homozygous for only one marker at a time. The 452 *y* females were found, upon testing, to include

174 nontransposes, 28 transposes, 15 *v–m* transposes (7 *v m*/++ and 8 *f car*/++), and 235 were indeterminate.

The 15 observed *v–m* transposes represent approximately 63 percent of the actual number of such transposes in the entire sample from the argument presented previously, which means that there must have actually been 24 such. This leaves 226 indeterminate nonhomozygous females (the 235 indeterminate *y* females less the 9 that were *v–m* transposes) to be divided into the nontranspose and transpose classes as 174 is to 28. These classes then become 195 and 31, respectively. In total, then there were observed 103 singly homozygous females and 83 (24 *v–m* transposes and 28 + 31 transposes) transposed and *v–m* transposed females.

Figure 2 shows the regions of exchange pertinent to this analysis, and table 2 gives the results from double exchanges according to region. From table 2, it can be seen (making the conventional assumptions about crossing over) that, whereas a precise determination of the relation between females homozygous for one marker at a time and transposes is not possible, from the double exchanges, there should be about two singly homozygous females recovered for each transpose, and approximately one singly homozygous female recovered for each *v–m* transpose. Taking into account the relative frequencies of transposes and *v–m* transposes, the expected recovery of singly homozygous females to transposed plus *v–m* transposed females from double exchanges (from the total of 186) becomes 115:71 as compared with the observed 103:83.

This agreement between the observed and the expected ratios, then, indicates that most, if not all, transposes are accounted for by double exchanges, and hence, single exchanges (half of which, in the *v* to *f* region, would produce transposed rings and none of which produce homozygosis), if they occur at all, must be rare.

On sister-strand crossing over

It has been shown (WEINSTEIN 1936; LINDEGREN and LINDEGREN 1937; SCHWARTZ 1953) that for chromosomes, other than rings, the expectations are the same whether it is assumed that crossing over takes place randomly between any two nonsister chromatids of a tetrad and that there is no sister-strand crossing over (the 'conventional' assumptions) or that crossing over occurs only between the two newly replicated strands (strands attached to the same centromere in the compound X chromosomes), with the probability of an effective sister-strand exchange in any region (i.e., an odd number of sister exchanges in the given region) being 1/2. For the

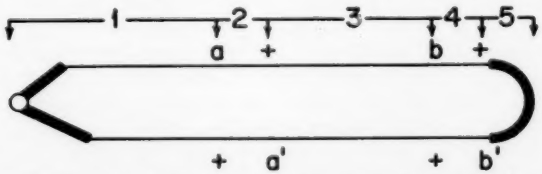

FIGURE 2.—The regions of exchange defined for those reversed compound rings that have been used to detect transposes of marker genes. The consequences of exchange in these regions are given in table 2.

TABLE 2

The expected relation between females homozygous for only one marker at a time, transposes, and v–m *transposes from exchanges of rank two*

Region involved	Products from	
	2-st-doubles	4-st-doubles
1, 2	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N
1, 3	2H: 1T: 1N	2H: 1T: 1N
1, 4	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N
2, 3	1H: 1$\hat{T}$: 2N	1H: 2T: 1$\hat{T}$
2, 5	2H: 1$\hat{T}$: 1N	1H: 1$\hat{T}$: 2N
3, 4	1H: 1$\hat{T}$: 2N	1H: 2T: 1$\hat{T}$
3, 5	2H: 1T: 1N	2H: 1T: 1N
4, 5	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N

The "markers" a, a', b, and b' represent, generally, the coupling relations in the rings carried by the parental females used for the transpose analysis. Double exchanges involving regions 1, 5 (since they result in nothing of interest here) and 2, 4 (since they must be very rare) are omitted; all 3-strand doubles result in anaphase II bridges and are, for this reason, omitted. H = detectable homozygote for one marker; T = detectable transpose; $\hat{T}$ = detectable v–m transpose; N = neither H, nor T, nor $\hat{T}$. (It should be noted that this class contains some multiple homozygous types from 1, 4 and 2, 5 exchanges, but it is not used in subsequent analyses.) The "regions involved" are shown in figure 2.

ring chromosomes, however, the expectations from the two sets of assumptions are different. The data from single ring X chromosomes in Drosophila (MORGAN 1933) are not consistent with the assumptions in a sister-strand analysis as outlined, but are consistent with the conventional nonsister-strand assumptions. The data from ring chromosomes in maize (MCCLINTOCK 1938, 1941; SCHWARTZ 1953), on the other hand, are consistent with the sister-strand analysis, but not with the other. It will be shown here that, as with single rings in Drosophila, the data from the reversed ring are not consistent with the assumption of sister-strand crossing over as just considered, but is consistent with the usual, nonsister strand assumptions. It should be emphasized that this applies only to sister-strand crossing over that follows the rules given here, and does not apply to the question of sister-strand exchange in general; this point will be developed in a later section.

The genetically distinct crossovers (sister and nonsister) in the reversed ring are shown in figure 3. The consequences of all combinations of these exchanges are given in table 3. A similar analysis, making the conventional assumptions about crossing over, is given in figure 4 and table 4. The first comparison of importance here depends on the fate of the equal (E) anaphase II bridges produced by crossing over. A bridge, in figures 3 and 4, has been designated equal if two strands that tie the sister centromeres separating at anaphase II together are of exactly the same length. This equality results if the heterochromatic regions labeled 1 and 2 in figures 3 and 4 are each present once in each of the two strands connecting the centromeres of a bridge. Bridges of this type have been shown to be excluded from the nuclei of the second meiotic division (NOVITSKI 1955) resulting, after fertilization by an X-bearing sperm (or an XY-bearing sperm in these experiments) in a patroclinous male. This is in

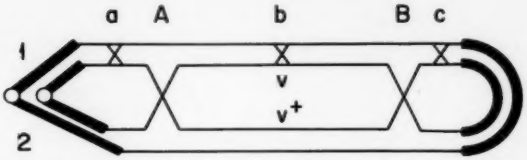

FIGURE 3.—The genetically distinct sister-strand (lower case) and nonsister-strand (upper case) exchanges in the reversed compound ring X chromosome. The numbers, 1 and 2, designate the two different heterochromatic regions. The genetic consequences of all combinations of these exchanges are given in table 3.

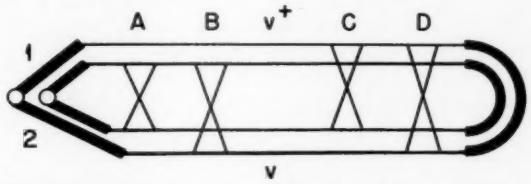

FIGURE 4.—The genetically distinct nonsister-strand exchanges in the reversed compound ring X chromosome. The consequences of all combinations of these exchanges are given in table 4.

contrast to anaphase II bridges generally (that is, either anaphase II bridges composed of only one strand, or unequal double-strand anaphase II bridges) that are lethal. Patroclinous males from this source are distinguishable from the regular males, which are the complementary products of the ring, because the parental females carry a marked homolog (FR2 carrying a normal allele of *y*) that the regular male class receives and are therefore non-*y*,*B*, but that the males from equal bridges do not receive and are therefore *y B*. Primary nondisjunction is one other predictable source of *y B* males. The complementary product to the *y B* males from primary nondisjunction is, however, the exceptional (i.e., y^+) female class. Although meiotic loss would be expected to result in some small excess of *y B* males over wild type females (SANDLER and BRAVER 1954), the observed excess (1,159 to 9; see table 1) is clearly much too large for this. It seems, therefore, most reasonable to suppose that all but a very small fraction of the exceptional males arise from equal bridges. The expected ratio of *y B* males to total homozygous females from double exchanges, making the conventional assumptions about crossing over, (figure 4 and table 4) is 1:1, with which the observed numbers 1,159 to 1,126 agree. This same expectation, assuming sister-strand crossing over (see figure 3 and table 3) is 3:2, with which the observations do not agree. Moreover, from the sister-strand analysis, it can be seen that one half the no-exchange tetrads should also produce patroclinous males (see table 3), which clearly are not recovered. This could also mean, of course, that sister-strand crossovers occur only in tetrads that also have nonsister exchanges.

Finally, it is possible to perform an analysis similar to that in figure 2 and table 2, to arrive at the expected ratio, from double exchanges, of singly homozygous females to females carrying transposed reversed rings, making the sister-strand assumptions. These expectations are approximately four singly homozygous females to one trans-

TABLE 3

The consequences of exchange in the reversed compound ring X chromosome of ranks 0, 1, and 2, supposing nonsister exchanges to occur between only the two "newly replicated" chromatids, with an effective sister-strand crossover occurring in any region with a probability of $\frac{1}{2}$

Rank and frequency	Crossovers involved		Products after anaphase II separation
	Nonsister	Sister	
$\frac{1}{2}$ E_0	None	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_0	None	a, b or c	Bridge (E)
$\frac{1}{2}$ E_1	A or B	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	A or B	a, b or c	Bridge ($\frac{1}{2}$ E + $\frac{1}{2}$ E′)
$\frac{1}{6}$ E_2	A and B	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{2}{6}$ E_2	A and B	a and c or a, b, and c	Reversed ring (*v*/*v*) + reversed ring (+/+)
$\frac{2}{6}$ E_2	A and B	b or a and b	Bridge (E′)
$\frac{3}{6}$ E_2	A and B	a or c or b and c	Bridge (E)

Exchanges have been omitted when such omission does not distort the relative frequencies of the distinct products given in the table. E_0 = no exchange, E_1 = single exchange, and E_2 = double exchange. E = equal and E′ = unequal. The "crossovers involved" are shown in figure 3.

TABLE 4

The consequences of exchange of ranks 0, 1, and 2, supposing no sister-strand crossing over in the reversed compound ring X chromosome. The "crossovers involved" are shown in figure 4

Rank and frequency	Crossovers involved	Products after anaphase II separation
E_0	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	A or D	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	B or C	Bridge (E′)
$\frac{1}{4}$ E_2	A and C	Bridge (E)
$\frac{1}{4}$ E_2	A and D	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{4}$ E_2	B and C	Reversed ring (*v*/*v*) + reversed ring (+/+)
$\frac{1}{4}$ E_2	B and D	Bridge (E′)

pose and about two singly homozygous females to one *v–m* transpose (as contrasted to 2:1 and 1:1 making the conventional assumptions). Although the data (103 homozygotes to 83 transposed plus *v–m* transposed rings) do not agree with these expectations, this argument alone would not be convincing since the nonagreement could be the result of some frequency of single exchanges. Taken, however, in conjunction with the argument from the equality of total homozygotes and patroclinous males, it may be considered further evidence for the inconsistency of the data with the expectations assuming sister-strand crossing over.

On the frequency of exchanges of ranks zero and two

From the foregoing, it would appear (1) that the frequency of single exchanges in reversed rings is either zero or very close to it, and (2) that the data from reversed rings are best dealt with assuming a conventional tetrad analysis. The frequencies of no-exchange and two-exchange tetrads may now be estimated. The appropriate analysis is given in figure 4 and table 4. The data have been presented in table 1. It can be seen that the total number of ring chromosomes and ring chromosome derivatives (i.e., homozygous rings and anaphase II bridges) from double exchanges is simply 4 times "total homozygous females," since, from table 4, one fourth of all the doubles yield homozygotes. This is 4,504 (4 × 1,126). For each homozygote from double exchanges, one nonhomozygous female is produced. There are, therefore, 1,126 of these, leaving 2,173 (3,299 − 1,126) nonhomozygous females that come from the no-exchange tetrad class (since singles are assumed not to occur). Now, if there were no other complications, the total number of ring chromosomes and ring chromosome derivatives accounted for (4,504 + 2,173 = 6,677) should equal the observed number of regular (*B*) males, since one half of all the eggs receive the homolog for the compound. However, from table 1, it is seen that 12,034 males were recovered as contrasted to only 6,677 ring products accounted for. That this is not a relative inviability of the ring classes is shown by the observed equality between total homozygotes (1,126) and patroclinous males (1,159). This is especially convincing since homozygous females are certainly somewhat less viable than heterozygous females and the *y B* males are probably more viable than the *B* males as the YSX.YL chromosome may be duplicated for the *y* region. It is clear, then, that there is some source of ring loss that is not accounted for by either inviability of the ring-bearing classes or the tetrad analysis. It will be shown in the next section of this report that this ring loss comes only from the no-exchange class. This being so, the frequencies of no-exchange and two-exchange tetrads may be estimated, the regular male class being used as the total. This is equivalent to increasing the no-exchange female class by the difference between the observed and the expected number of regular males. The frequency of tetrads of rank two then becomes 0.37 (4,504/12,034), whereupon no-exchange tetrads must have a frequency of 0.63. It is also possible to estimate the recovery of rings from no-exchange tetrads; this is 2,173/7,530 = 0.29; that is, only 29 percent of the no-exchange tetrads are recovered.

The frequencies of exchange arrived at here are rather interesting since they are very similar to the exchange frequencies in reversed acrocentric compound X chromosomes (SANDLER 1954). The best comparison would come from computing exchange values for the reversed acrocentric by the same method that is used here for the reversed ring. In the report on the reversed acrocentric, the single exchange frequency was estimated from the difference between males and females recovered, assuming no viability differences between compound-bearing females and regular males. It might also be mentioned that the calculations of exchange values in that work contain a minor error in that one lethal class was not considered; the corrected values are, however, very close to those given and do show a value for the frequency of single exchanges of very close to zero (actually about 12 percent). A typical set of data

from a cross of females carrying reversed acrocentric compound X chromosomes (line ND 34 in table 10; SANDLER 1954) and FR2 by YSX.YL, *y B* males are:

Regular ♂♂ = 2,925
Nonhomozygous ♀♀ = 1,836
Homozygous ♀♀ = 652.

The male class has been corrected for meiotic loss, the homozygous female class has been corrected for those homozygous for wild type alleles, and the nonhomozygous female class has been corrected accordingly. On the assumption that single exchanges occur with a frequency of zero, double-exchanges become 0.45 (2 × homozygous females/regular males = 1,304/2,925), whereupon no-exchange tetrads must have a frequency of 0.55. The tetrad analysis is somewhat different from that for the reversed ring, and the appropriate analysis is given in SANDLER (1954).

This comparison of $E_0 = 0.55$ and $E_2 = 0.45$ for the reversed acrocentric and $E_0 = 0.63$ and $E_2 = 0.37$ for the reversed ring suggests that the rate of exchange in the two compounds is the same.

On the effect of FR2 on exchange

It has been shown (SANDLER 1954) that the rate of homozygosis in reversed acrocentric compound X chromosomes is markedly decreased if the parental compound-bearing females do not carry a Y chromosome (or FR2) as a homolog for the compound. To test for this phenomenon in reversed rings, females carrying reversed rings heterozygous for *m* and *v*, with no homolog for the compound were crossed to YSX.YL, *y B* males with no homolog. The progeny included:

y B ♂♂ = 684 (814)
Total ♀♀ = 243
y v ♀♀ = 15
y m ♀♀ = 13.

These results may be compared with those given in table 1. The appropriate figures here are:

Regular (*B*) ♂♂ = 10,113 (12,034)
Exceptional (*y B*) ♂♂ = 974 (1,159)
Total ♀♀ = 4,425
y v ♀♀ = 469
y m ♀♀ = 453.

The numbers in parentheses are the observed numbers corrected for meiotic loss.

To compare the rates of homozygosis in these two experiments by computing the "percentage homozygosis" as "number of homozygous females" divided by "total females," as may be done for the reversed acrocentric, would lead to spurious results if there were a difference in exchange frequency between the two experiments, since only 29 percent of the no-exchanges result in recovered females (see preceding section) whereas, from the tetrad analysis, 50 percent of the double exchanges are recoverable.

To circumvent this difficulty, the observed number of males (regular plus exceptional) will be used as the total. This number will be somewhat affected by exchange frequency since the exceptional male class is a double-exchange product, but since such males represent only a small proportion of the total number of males, this should have little effect on the results. In any case, this error would tend to obscure a difference between the two experiments. The exceptional males must be included in the total because, in the experiment in which the parental females did not carry FR2, these males are not distinguishable from the regular males. The comparison, then, for the case of homozygosis for *v*, becomes 4.2 (469/10,113 + 974) for the case in which the females carried FR2 and 2.2 (15/684) for the case in which they did not. It is clear that this same difference would manifest itself if homozygosis for *m* were measured. It appears, then, that the effect of FR2 on exchange (the rate of homozygosis being used as a direct measure of exchange) does extend to reversed rings.

Since this is so, very strong evidence may be presented indicating that the deficiency in recovery of ring-bearing females observed in the preceding section comes exclusively or almost exclusively from the no-exchange tetrad class. This may be done most simply by predicting the sex ratio in the experiment in which the compound-bearing females did not carry a homolog from the results of the experiment in which they did, assuming that only 29 percent of the no-exchange tetrads are recovered and that double exchanges are one half as frequent when the compound-bearing females do not carry FR2 as when they do. Arithmetically, this may be done as follows: from each 100 eggs, at the first meiotic division, 50 will receive a compound ring and 50 will not. All 50 of those that do not receive the ring result in males (since meiotic loss of the YSX.YL chromosome has been corrected for, the fact that only nullo-X eggs being fertilized by YSX.YL-bearing sperm are recovered and only ring or ring-derivative eggs being fertilized by nullo-XY sperm are recovered may be ignored); of the 50 that do receive the compound ring, 9.3 will have two exchanges (since E_2 here is 0.185 or $1/2 \times 0.37$) and the rest, 40.7, will have no exchanges; from the 9.3 with two exchanges there will be recovered 4.6 females and 2.3 males (see table 4); from the 40.7 with no exchanges, 29 percent, or 11.8, are recovered as females. In total, then, there should be 52.3 males and 16.4 females recovered or 3.2 males per female. By the figures corrected for meiotic loss given, the ratio of males to females is 3.3 (814/243). The data will not agree with expectations arrived at if the loss of rings is assumed to come at random from both the zero-exchange and the two-exchange tetrads.

On the distribution of exchanges along the length of the chromosome

It has been shown here that reversed acrocentric and reversed ring compound chromosomes behave very much alike. One further comparison between these two compounds is possible—a comparison of the distribution of exchanges along the length of the chromosome. This is most readily done by comparing the rates of homozygosis for individual markers among all homozygotes in the reversed acrocentric and reversed ring compounds. This comparison is given in table 5. It can be seen that the distribution of exchanges is very similar for the two compounds.

TABLE 5

A comparison of the relative rates of homozygosis in the reversed ring as compared with the reversed acrocentric compound X chromosome. Percentage homozygosis is computed as the number of homozygotes for a given allele over the total number of homozygous alleles

Allele	Reversed acrocentric homozygotes		Reversed ring homozygotes	
	Number	Percent	Number	Percent
cv	70	12	182	17
v	215	38	469	42
f	177	31	301	27
car	110	19	155	14

Data for the reversed acrocentric are from SANDLER (1954). The data, in both cases, were collected from females carrying a homolog for the compound chromosome.

DISCUSSION

A comparison of reversed acrocentric and reversed ring compounds

The reversed ring and the reversed acrocentric have been shown to behave alike according to all the criteria applied. Both compounds show the same effect on crossing over of a heterochromatic homolog for the compound, both show the same distribution of exchanges along the length of the chromosome, both show a reduction (or an absence) of single exchanges, and both show about the same frequencies of zero-exchange and two-exchange tetrads. They differ, in fact, only in that the reversed ring is recovered less often from no-exchange tetrads than expected, whereas no such deficiency is manifest in reversed acrocentric data. It now seems very likely that both the lack of single exchanges and the effect on crossing over of the Y chromosome, or FR2, (and, possibly also, as will be developed presently, the deficiency of rings from reversed ring-bearing parents) are different manifestations of the same cause. The absence of single exchanges is, of course, the most striking feature to be considered.

It would seem that, with respect to the lack of singles (and perhaps the other effects), there are only three possibilities: (1) that single exchanges are occurring, but that the crossover products are not recovered for some reason, giving the impression of an absence of singles; (2) that single exchanges are occurring, but that one or more of the assumptions that goes into the tetrad analysis is incorrect, with the result that single exchanges appear, from the analysis, not to be occurring; and (3) that there are in fact no singles occurring, which means that there exists some property or properties of chromosomes, that under ordinary conditions does not manifest itself, but that, under the structural conditions imposed on the chromosomes in reversed acrocentric and reversed ring compounds results in the aberrant behaviors noted. A comparison of reversed acrocentric, reversed ring, and attached-X compounds suggests that the structural condition in question is the interstitial heterochromatic segment. The first two of these possibilities were considered in some detail previously (SANDLER 1954), and were shown to be inadequate to explain the data from reversed acrocentrics. These same arguments, in general, apply also to reversed rings, by extrapolation if nothing else. There is, in addition, one rather general consideration that also tends to eliminate these possibilities. The fact that

the effect of a Y chromosome (or FR2) on crossing over manifests itself in both the reversed acrocentric and reversed ring compounds suggests that this effect and the absence of single exchanges are two indications of the same phenomenon. If, moreover, the deficiency of rings recovered from reversed ring females is not a consequence of interlocking, as it may very well not be, it seems reasonable that this too is a result of the same cause. If this is so, then it is certainly very difficult to see how either a lack of recovery of certain products or an error in the assumptions made in the tetrad analysis would be sufficient to account for all these phenomena.

One final point in this connection is of some consequence. From the results of the reversed acrocentric compound alone, it would be possible to conclude that some of the tetrads do not pair (the difficulty in synapsis being presumably caused by the reversed acrocentrics having to fold back on themselves in order to pair) and that this proportion has, of course, no exchanges. When, however, the compound does pair, then there are only double exchanges. The results from the reversed ring would seem to deny this possibility since the reversed ring should pair with the same ease as an attached-X. It seems necessary to conclude, therefore, that even though the component chromosomes of the compounds are paired with each other, they may either not cross over at all or they may cross over twice (or possibly more than twice).

Sister-strand crossing over

One of the specific results of this analysis of the reversed compound ring X chromosome is the inconsistency of the data with the supposition that crossing over occurs initially between only two of the four nonsister chromatids with sister-strand exchange then randomizing the exchange regions. This conclusion from the reversed ring agrees with that based on the data from single ring X chromosomes in Drosophila (MORGAN 1933). It has, on occasion, been argued that the nonagreement of the results from single ring X chromosomes with the expectations based on a sister-strand analysis may be interpreted as coming about because the ring is small and, if this results in a certain rigidity, each twist in the ring might be automatically accompanied by a second twist that, if a sister-strand exchange occurs at each twist, will result in an even number of sister exchanges in every tetrad. Such a situation would, of course, give results indistinguishable from those if no sister-strand crossing over takes place. Such an argument, however, based on the relatively small size of single rings, does not apply to the reversed ring since it appears to be a rather large ring in larval neuroblasts and in genetic length.

There is, perhaps, one qualification that ought to be considered. If, for any reason, only even numbers of sister-strand exchanges per tetrad could occur in the reversed ring, then the results from the reversed ring would be in agreement with the expectations (since, indeed, the experimental distinction between the conventional, as opposed to the sister-strand, hypothesis depends wholly on the occurrence of tetrads with an odd number of sister exchanges). The reason for suggesting this particular qualification is that one way of describing the observed absence of single exchanges in the reversed ring is to say that there can occur only an even number of nonsister exchanges. If this is so, then the possibility of only an even number of sister exchanges might be acceptable. Moreover, if this is so, then the difference between maize and

Drosophila, with respect to crossing over, is simply that in Drosophila sister exchanges always occur in pairs; a distinction much less profound than one that supposes that sister-strand crossovers never occur in Drosophila but are, in maize, a regular and essential part of the process of crossing over.

Possible interlocking of rings

It has been shown that although the data from reversed rings and from reversed acrocentrics suggest that the two compounds behave very much alike, they do differ markedly in one particular: whereas all the expected reversed acrocentric compounds are recovered from reversed acrocentric compound-bearing females, only 29 percent of the expected compound rings from no-exchange reversed ring tetrads are recovered, although all the expected products from double exchanges are observed. It has been shown, moreover, that this deficiency in rings cannot be attributed to inviability of the ring-bearing classes. Certainly, one possible explanation of this discrepancy is that some fraction of ring tetrads give rise to mutually interlocked ring complexes that result in lethal zygotes. If this is the proper explanation of the deficiency in the recovery of rings, then, surprisingly enough, it means that the rings from no-exchange tetrads are being lost by interlocking, which strongly suggests that crossing over is a process whereby the interlocked complexes may be resolved (a conclusion similar to that of MATSUURA 1940). The question that immediately suggests itself is how tetrads in which no exchange has occurred may become interlocked. There seem to be but two possibilities. Either (1) the process of replicating a new strand does not occur on only one surface of the original strand so that the new chromatid, when completely formed, is wound around the original chromatid, or (2) that sister-strand exchanges are occurring. The data collected to date are not sufficient to distinguish between the two possible origins of the interlocked complexes.

There is, however, one argument that ought to be mentioned suggesting that this deficiency of rings has an explanation other than that of elimination by interlocking. If there is some lethality from interlocking, then this lethality could be visualized as resulting from either the breakage and subsequent lethality of interlocked rings, or a lethality caused by the anaphase bridge-like structure of the unresolved interlocked complexes. If the former concept were the proper one, then it would seem that the breakage of one of the two interlocked rings would be sufficient to resolve the complex, which would mean that the observed lethality (70 percent of the no-exchange tetrads) actually represents only half the instances of interlocking. This supposition will clearly not fit the data. The alternative idea—that the interlocked complexes remain intact with the result that all instances of interlocking are lethal—will fit the data numerically; but this idea leads to a mechanical difficulty. It is difficult to see why such a complex would be lethal since it is structurally the same length as the equal anaphase II bridges that result, not in lethality, but in detectable nullo-X eggs.

Although these arguments are most certainly not conclusive, they do suggest that the observed deficiency of rings may not be a reflection of interlocking. If this is the case, then (1) these data represent evidence that chromosomes have some mechanism that enables them either to keep from becoming interlocked when they are struc-

turally rings, or to extricate themselves from the complex after becoming interlocked, and (2) it then seems reasonable to suppose that this observed discrepancy is somehow related to the other atypical meiotic behaviors of the reversed ring. One such relation, for example, is as follows. It may be imagined that in the reversed ring all the products of single exchanges are lethal. In the present analysis, this would appear as if the lethality occurred in the no-exchange class. This interpretation, although acceptable for the case of the reversed ring, would not be applicable to the reversed acrocentric, since there is no lethality unaccounted for in that compound.

SUMMARY

A genetic analysis has been made of a reversed compound ring X chromosome. This compound is structurally like an attached-X with its free ends connected by a heterochromatic segment or, which is the same thing, it is similar to a reversed acrocentric compound X chromosome with its free end attached to the centromere by a heterochromatic segment.

The analysis revealed the following points:

1. The data are found to be inconsistent with the assumption that crossing over takes place between only two nonsister chromatids (the "newly replicated" chromatids), with sister-strand crossing over then randomizing the exchange regions, and are found to be consistent with the conventional assumptions made about the nature of crossing over.

2. The compound ring behaves very much like the reversed acrocentric compound X chromosome in that (1) the presence of a Y chromosome, as a homolog for the compound, in the parental females approximately doubles the rate of exchange (as measured by the rate of homozygosis) and (2) the frequency of single exchanges is very nearly, or perhaps actually, zero although there is a high frequency of both no-exchange and two-exchange tetrads. These questions are discussed in text.

3. Unlike reversed acrocentric compounds, the reversed ring is not recovered as often as expected. It is shown that whereas all the products of the two-exchange class are observed, only 29 percent of the no-exchange tetrad class is recovered. The possibility of this depression in the recovery of reversed rings being caused by ring loss by virtue of the formation of mutually interlocked ring complexes is considered.

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SEGREGATION IN NEW ALLOPOLYPLOIDS OF GOSSYPIUM. II. TETRAPLOID COMBINATIONS

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IN two other papers in this series (GERSTEL 1956; GERSTEL and PHILLIPS in prep.) the segregation of artificial hexaploid combinations was described. These had been obtained by crossing tetraploid New World cottons with diploid Wild American species and subsequent doubling of the chromosome number. For certain characters these hexaploids were of duplex genotype (i.e. of the type *ZZzz*). Segregation occurred in the crosses with recessive testers, yet the resulting ratios were wider than the 5:1 ratio expected from random chromosome association. The results were interpreted as due to weakly preferential associations at the prophase of meiosis between chromosomes derived from the same parent species. The present paper deals with allotetraploids synthesized from various diploid members of the genus. These were either taken from the same section as in the cases of 4n(*G. arboreum* × *herbaceum*) and 4n(*G. thurberi* × *raimondii*) or from different sections as in 4n (*G. arboreum* × *anomalum*) and 4n(*G. arboreum* × *thurberi*). In several instances, it was possible to compare the segregation ratios for factors located on different chromosomes.

MATERIALS AND METHODS

The diploid (n = 13) species belong to four sections (HUTCHINSON, SILOW and STEPHENS 1947), *viz.*: Herbacea, the largely cultivated Old World cottons *G. arboreum* and *G. herbaceum* which carry the A genome of chromosomes; wild African Anomala (*G. anomalum*) with the B genome: Klotzschiana (*G. raimondii*) and Thurberana (*G. thurberi*). The latter two sections comprise Wild American species with D genomes.

Amphiploids were synthesized from seedlings of artificial hybrids by SCHWANITZ's (1949) tragancanth method and maintained in a vigorous and actively growing state while being used for testcrossing. In designating amphiploids the name of the female parent of the original hybrid will hereafter always be written first. Most of the parental crosses can be made in both directions but generally the one most readily performed under Raleigh conditions was selected. The colchicine treated hybrids were verified as tetraploids by cytological analysis of pollen mother cells. The number of amphiploid plants of a given type ranged from one to three with two individuals in most cases. The results from different amphiploids of identical parentage will be presented together as they were found to behave similarly with

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respect to segregation. In these amphiploids, lines were combined which differed in various marker alleles; the resulting tetraploids were, therefore, of the duplex type. Most of the lines had been introduced to our institution by STEPHENS, and had been previously described in various publications (STEPHENS 1951, 1954; GERSTEL 1953, 1956). With respect to the alleles employed in the present study, the constitution of these lines is explained below. The symbols for the cultivated species are those of the publications by STEPHENS; those for wild species are suggested by the authors:

G. arboreum:

S4004: r_2^{OGO} (green plant body and no petal spot); P_a (yellow pollen); y_aY_c (white petal, complementary yellow with New World cottons); l (broad leaf).

S4065: R_2^{RGS} (red plant body and small petal spot); P_a; L^N (narrow leaf).

S5000: rl (no red lethal); r_2^{OOO} (similar to r_2^{OGO}; absence of "ghost" component irrelevant here); P_a; y_aY_c; ne (no foliar nectaries).

S5133: r_2^{OGO}; y_aY_c; L^L (laciniate leaf); ne.

"Sanguineum": Rl_a (red lethal complementary); R_2^{RGS}.

G. herbaceum:

S5005, Gillet's Iraqui, *africanum*, *kuljianum*: rl; R_2^{OGS} (green plant body and small petal spot); P_a; Y_aY_c (yellow petal); l; Ne (foliar nectaries). The second of these lines is called "Iraqui" in the tables for short.

Tashkent cream pollen: as above, but p_a (pale pollen). Called Tashkent in tables.

G. anomalum: rl; R_2^{ANO} (green plant body and "*anomalum* ghost", a large petal spot complementary); p_a; $Y_aY_c^p$ (pale petal); L^A (*anomalum* lobed leaf).

G. thurberi: l^{THU} (deeply lobed leafshape of incomplete dominance—called L^u by GREEN, 1953, after transfer to *G. hirsutum*). Carries also recessive allele at the R_1 locus (green plant body and weak petal spot: RHYNE, 1955) and cream pollen.

G. raimondii: R_1^{RAI} (very large petal spot and deep anthocyanin coloration of anther filaments).

For testing segregation of the artificial allotetraploids both natural tetraploids (*G. hirsutum*, *G. barbadense*) and an artificial autotetraploid were used. The latter, 4n *G. arboreum* S5000, was the only type suited for testing corolla color segregation in 4n(*G. arboreum* × *herbaceum*) because of the presence of a complementary yellow gene for Y_a in the New World cottons. It also was useful as a tester for most other contrasts, except for P_a and Rl_a, having yellow pollen and not carrying the complementary Rl_b (see genic formula for S5000 above). However, because the fertility of this autotetraploid was very low, it was used only rarely. More widely employed were various lines of *G. hirsutum* (Coker, Stoneville, S5082, etc.), which contained Rl_b and therefore tested for the red lethal complementary Rl_a. They also were suitable testers for pollen color, leaf shape, plant body color and petal spot segregations. The spotless r_2^{OGO} type of *G. arboreum* gave with *G. hirsutum* a complementary small petal spot which could not be safely distinguished from the Old World spot produced by R_2^{RGS}, but presence or absence of the large *anomalum* spot complementary could readily be scored. Many of the offspring from crosses between amphiploids having the red lethal factor Rl_a with lines of *G. hirsutum* containing the complementary Rl_b died before other characters could be identified. Testing for the segregation of other

characters in these Rl_a amphiploids was accomplished by employing suitable *rl* genotypes: 4n *G. arboreum* S5000, the Lacandon variety of *G. hirsutum* and a *G. barbadense* line (Hallmark). All these lines were recessive with respect to the characters they were used to test. Another *G. barbadense* line (TZVR) with *rl* and green plant body also was used.

Pollination of New World flowers with pollen from 4n(*G. thurberi* × *raimondii*) usually resulted in empty seeds, but this allotetraploid could effectively be tested as a male parent with the artificial hexaploid combination 6n(*G. hirsutum* × *thurberi*) as female.

In order to conserve space the various lines employed in testing were not named in the tables, where only the tested amphiploids but not the recessive testers were specified.

RESULTS

1. 4n(G. arboreum × herbaceum)

Amphiploids of the two cultivated Old World species were tested in several combinations for a total of five characters (table 1). Three of these were known to be inherited independently; namely R_2^{RGS}, V_a and L^N (HARLAND, 1939), while the linkage relationships of Rl_a and P_a with each other and the other three loci were hitherto undetermined. (One progeny, in row 5 of table 1, lent itself to an analysis of pollen

TABLE 1

Segregation of 4n(G. arboreum × herbaceum)

	Red Lethal (Rl_a)*		Plant body (R_2^{RGS})			Corolla (V_a)		Pollen (P_a)		Leaf shape (L^N)	
	Lethal	Viable	Red	Interm.	Green	Yellow	White	Yellow	Pale	Narrow	Broad
a) Amphiploid as ♀											
4n(*G. arboreum* Sanguineum × *herbaceum* Tashkent)	39	14	3	7	2						
4n(*G. arboreum* Sanguineum × *herbaceum* Iraqui)	80	24	3	7	5						
4n(*G. arboreum* Sanguineum × *herbaceum* r. *africanum*)	9	2	0	5	4						
4n(*G. arboreum* Sanguineum × *herbaceum* S5005)	140	28	4	11	6						
4n(*G. arboreum* S4065 × *herbaceum* Tashkent)			4	41	9			42	12	49	5
4n(*G. arboreum* S5000 × *herbaceum* Tashkent)								9	1		
Total 4n as ♀	268	68	14	71	26			51	13	49	5
b) Amphiploid as ♂											
4n(*G. arboreum* Sanguineum × *herbaceum* S5005)	41	10	2	33	8	36	7				
Overall total	309	78	16	104	34	36	7	51	13	49	5
P(5:1 or 1:4:1)	.1–.05		.05–.02			.95–.90		.5–.1		.2–.1	

* Includes data published previously (GERSTEL 1954).

TABLE 2

Independence test for plant body color, leaf shape and pollen color loci from 4n (G. arboreum *S4065* × herbaceum *Tashkent*) × *recessive* G. hirsutum. (*S4065* = RLP*; *Tashkent* = rlp)

					Total	P
Exp. (25:5:5:1)†	37.5	7.5	7.5	1.5	54	
Observed:						
Plant body—Leaf shape	41 *RL*	4 *Rl*	8 *rL*	1 *rl*	54	.7–.5
Pollen—Leaf shape	38 *PL*	4 *Pl*	11 *pL*	1 *pl*	54	.5–.3
Plant body—Pollen	35 *RP*	10 *Rp*	7 *rP*	1 *rp*	54	.8–.7

* Abbreviated gene symbols.

† Based on random association, random chromosome disjunction, complete dominance and equal survival.

color linkage relationships. The result, suggesting independence of the pollen color locus from both plant body color and leaf shape, is presented in table 2.) In the main, the data of table 1 were obtained from testcrosses in which the artificial amphiploids served as female parents (part a, table 1) but some reciprocal crosses were made (b) and the results were combined since they did not differ materially. In four out of five cases the data conformed with the 5:1 gametic ratio expected from random behavior at meiosis of four homologous chromosomes represented by the *ZZzz* model used previously. With respect to the fifth case, plant body color, dominance was incomplete and three progeny classes could be distinguished: deep red, intermediate and green. These classes presumably were produced by gametes with two, one and zero R_2^{RGS} alleles, respectively. The fit to a 1:4:1 ratio was poor in this case and will be considered later.

In some cases the number of plants available was very small and in that respect the data must be regarded as preliminary. However, it is clear that there was no tendency for the ratios to be *wider* than 5:1; i.e., there was no evidence for preferential associations between chromosomes derived from the same parent species. On the contrary, some ratios were notably *narrower* than 5:1, which will be discussed below.

Table 3 contains the results from backcrosses of diploid *G. arboreum* × *herbaceum* hybrids to multiple recessive *G. arboreum* testers. The reason for making these crosses will be given in the Discussion together with an interpretation of the data.

2. *4n*(G. thurberi × raimondii)

Another amphiploid between two species carrying similar genomes gave a considerably wider ratio than that expected from random segregation. The amphiploid from the two D genome species *G. thurberi* and *G. raimondii* was tested as a male parent on a hexaploid *G. hirsutum* × *thurberi*. This hexaploid segregated for various characters, but since its component species lacked the large petal spot and pigmentation of the anther filaments characteristic of *G. raimondii*, segregation at the R_1^{RAI} locus in 4n *G. thurberi* × *raimondii* could be tested against the background of the hexaploid. A ratio of 363 R_1^{RAI} spotted to 27 unspotted plants or 13.4:1 resulted (table 4). The cross between 4n(*G. thurberi* × *raimondii*) as a female parent and *G.*

TABLE 3

The result of backcrosses of diploid F_1 G. arboreum × herbaceum *to a multiple recessive* G. arboreum *tester*

a.) Monofactorial segregations. Alleles* from the *herbaceum* parent are listed in the left column in each case.

Hybrid parent	*l* · *L*	*Y* · *y*	*R* · *r*	*Ne* · *ne*
F_1 (S5133 × *kuljianum*) ♂	94 · 76	66 · 103	76 · 93	94 · 76
F_1 (S5000 × S5005) ♂	33 · 37	36 · 34	37 · 33	29 · 41
F_1 (S5133 × *kuljianum*) ♀	14 · 16	14 · 16	16 · 13	13 · 8
	141 · 129	116 · 153	129 · 139	136 · 125
χ^2 (1:1)	0.533	5.089	0.373	0.464
P (1)	.3–.5	.02–.05	.5–.7	.5–.7

b.) Frequencies of plants carrying various numbers of *G. herbaceum* alleles.

Hybrid parent	Number of *G. herbaceum* alleles					
	0	1	2	3	4	Total
F_1 (S5133 × *kuljianum*) ♂	11	45	68	31	14	169
F_1 (S5000 × S5005) ♂	4	23	21	18	4	70
F_1 (S5133 × *kuljianum*) ♀	1	3	10	5	2	21
	16	71	99	54	20	260
Exp. $(\frac{1}{2} + \frac{1}{2})^4$	16.25	65.00	97.50	65.00	16.25	260
χ^2	0.004	0.554	0.023	1.861	0.865	3.307
P (4)	—	—	—	—	—	.5–.7

* Gene symbols are abbreviated.

TABLE 4

Segregation of 4n(G. thurberi × raimondii) *as male parent*

Plant no.	*raimondii* spot	No *raimondii* spot
3-129-A	175	14
3-129-B	35	3
3-129-C	153*	10
	363	27

* One plant was extremely abnormal with deformed leaves and petals and showed R_1^{RAI} spots in some flowers, but not in others.

hirsutum is possible and should permit scoring not only for R_1^{RAI} but also for leaf shape segregation. Unfortunately only a few plants have been obtained to-date because of a strong tendency of the artificial amphiploid to shed its capsules.

3. Amphiploids between Old World cottons and G. anomalum

Segregation data from *G. arboreum* × *anomalum* and *G. anomalum* × *herbaceum* amphiploids are presented in tables 5 and 6. The figures show a very low amount

TABLE 5

Segregation of 4n (Old World × anomalum)

	Red lethal (Rl_a)*		Plant body (R_2^{RGS})			Petal Spot (R_2^{OGO})		Corolla (Y_c)		Pollen (P_a)		Plant features	
	Lethal	Viable	Red†	Interm.	Green	Large	Small	Yellow	Cream	Yellow	Pale	Norm.	Reduced
a) Amphiploid as ♀													
4n(*G. arboreum* Sanguineum × *anomalum*)	189	4	2	59	0			33	0				
4n(*G. arboreum* S5133 × *anomalum*)						98	1	99	0	98	1	98	1
4n(*G. arboreum* S4004 × *anomalum*)						110	4	113	1	111	3	110	4
4n(*G. herbaceum* r. *kuljianum* × *anomalum*)						52	1	53	0	52	1	53	0
Total 4n as ♀	189	4	2	59	0	260	6	298	1	261	5	261	5
b) Amphiploid as ♂													
4n(*G. arboreum* Sanguineum × *anomalum*)	128	3	3	108	1			56	1	55	0	55‡	0
4n(*G. arboreum* S5133 × *anomalum*)						56	0	55	1	55	1	56	0
4n(*G. arboreum* S4004 × *anomalum*)						8	0	7	1				
4n(*G. herbaceum* r. *kuljianum* × *anomalum*)						51	0	30	1	34	0	34	0
Total 4n as ♂	128	3	3	108	1	115	0	148	4	144	1	145	0
Overall total	317	7	5	167	1	375	6	446	5	405	6	406	5
Heterogeneity§	Chi square = 4.0796; df = 7; P > .7												

* Includes data published previously (GERSTEL 1954).

† Plants in this row also had small petal spots, with one exception.

‡ 2 highly abnormal, dwarfish plants not counted.

§ For tables 5 and 6 combined.

of segregation; all together there were listed, in tables 5 and 6, 2525 phenotypes due to two or one dominant alleles (*ZZz* and *Zzz*, where the last symbol represents the allele contributed by the recessive tester) and 33 recessives (*zzz*); i.e., a 76.5:1 ratio. All the individual segregation frequencies could be fitted to this total as indicated by heterogeneity test. The classes with two dominant alleles (*ZZz*) which could be recognized in three of the cases did not deviate significantly from this ratio either (P>.05 in all three instances).

Some of the loci investigated were the same as in the *G. arboreum* × *herbaceum* amphiploids, namely Rl_a, R_2, P_a and *L*. Besides, the Old World × *anomalum* amphiploids also segregated for the recessive Y_c^p allele of *G. anomalum*. Segregation at the anthocyanin locus was listed in two separate columns under "Plant Body" and "Petal Spot" depending on the markings of the Old World cotton parent. Similarly, leaf shape segregations were of two different types, depending on whether the Old

TABLE 6

Leaf shape segregation of 4n(Old World × anomalum)

	Laciniated $L^L L^L_l$	Intermediate $L^L L^{ANO}_l$	*Anomalum* like $L^{ANO}_{ANO_l}$	Intermediate L^{ANO}_{ll}	Broad ll
a) Amphiploid as ♀					
4n(*G. arboreum* S5133 × *anomalum*)	0	98*	1		
4n(*G. arboreum* S4004 × *anomalum*)			0	112*	1
4n(*G. herbaceum* r. *kuljianum* × *anomalum*)			0	52	1
b) Amphiploid as ♂					
4n(*G. arboreum* S5133 × *anomalum*)	1	55	0		
4n(*G. arboreum* Sanguineum × *anomalum*)			0	53	0
4n(*G. herbaceum* r. *kuljianum* × *anomalum*)			0	34†	0
Total from $L^L L^L L^{ANO} L^{ANO}$ amphiploids	1	153	1		
Total from $llL^{ANO}L^{ANO}$ amphiploids			0	251	2
Heterogeneity‡	Chi square = 4.0796; df = 7; P > .7				

* One plant in these classes deviated toward $L^{ANO}L^{ANO}_l$ and may have been misclassified.

† One plant with totally abnormal leaf conformation and chlorophyll deficient streaks not counted.

‡ For tables 5 and 6 combined.

World species contributed the laciniated or the broad allele to the amphiploid parent. The loci for L and R_2 (HARLAND 1939) and also V_c and P_a (STEPHENS 1954b) are known to be independent. Evidence was presented in table 2 for the independence of P_a from both L and R_2. The results from the amphiploids with *G. anomalum* bore this out: the same families in tables 5 and 6 which segregated for pollen color segregated also for petal spot, corolla color, plant features and leaf shape. Yet none of the individual segregants with pale pollen had small petal spot, cream corolla, reduced features or either of the rare leaf types. The 25 plants listed as being of the recessive type for these characters and the one plant with deeply laciniated leaves in these three families were 26 different individuals. Thus, there is a strong presumption of genetical independence of all these loci.

The relationships of the Rl_a locus are still unknown since the families segregating for it were not scored for any other character. Besides those mentioned, an additional plant type turned up in the cultures that differed characteristically from normal in that the vegetative features were appreciably reduced in size; the repeated occurrence of this character with a frequency comparable to other segregations suggested the existence of a complementary interaction between a *G. anomalum* gene and the *G. hirsutum* tester parents. The five segregants with reduced features may be presumed to have carried the *G. anomalum* allele in double dose. Further investigation of this point is needed, of course.

4. G. arboreum × thurberi *amphiploids*

Amphiploids between Asiatic *G. arboreum* and American *G. thurberi* exhibited almost complete stability (table 7). In this case three dominant (or epistatic?) alleles

TABLE 7

Segregation of 4n(G. arboreum *S4065* × thurberi)

	Plant body (R_2^{RGS})		Pollen (P_a)		Leaf Shape (L^N)	
	Red	Green	Yellow	Pale	Narrow	Broad
Amphiploids as ♀	158	0	118	0	117	1

were introduced from *G. arboreum*, R_2^{RGS}, P_a and L^N. All progeny plants from testcrosses with *G. hirsutum* were uniformly red and those that bloomed had yellow pollen. One plant was distinguished by its broader leaf shape from the remainder; it apparently lacked the L^N allele.

DISCUSSION

The question must be raised in a study of this kind whether the observed segregation ratios accurately reflect the events of early meiosis or whether they are secondarily modified by selective elimination of chromosomes, gametes and zygotes. If the latter were the case, it would detract heavily from the value of the method for a quantitative estimation of preferential chromosome affinity.

Indeed, a great amount of elimination of gametes did occur in all these amphiploids. SARVELLA (1956) has given estimates of pollen abortion for them which ranged from 35.5 percent in 4n(*G. thurberi* × *raimondii*) to 84.8 percent in 4n(*G. arboreum* × *herbaceum*). Similarly, the number of fertile seeds in each of the amphiploids was only a fraction of the number of ovules per capsule. Zygote elimination could also be observed. A small number of seedlings which never grew past the cotyledonary stage were found in most cultures and could not be taken in account in scoring. In addition, some rare seedlings died very early or even failed to emerge. The question whether all this elimination was at random with respect to the genetic markers under observation or not can be attacked only indirectly by comparing reciprocal crosses and by comparing the sizes of the segregating classes produced by *ZZ* and *zz* gametes. In a previous paper, dealing with *G. hirsutum* × *raimondii* and *G. hirsutum* × *thurberi* allohexaploids, the conclusion was reached by these means that postmeiotic selection affected certain segregants but was not of sufficient magnitude to obscure the far greater differences in the meiosis of the two hexaploids (GERSTEL 1956).

In the present study reciprocal crosses of some of the amphiploids were made (tables 1, 5 and 6) but the data hardly sufficed for statistical comparisons. The numbers of plants produced by *ZZ* and *zz* type gametes could be compared only in a few instances where *ZZz* type plants could be distinguished from *Zzz*; these favorable cases will be discussed further on.

Chromosome elimination could be studied in the aforementioned *G. hirsutum* × *raimondii* hexaploids because of rather precise dosage effects which made possible the recognition of offspring which had more or less than their share of petal spot alleles. It appeared that most gametes produced by the hexaploids had resulted from regular two-by-two disjunction with only a negligible fraction containing one

or three alleles. The genes used in the present investigation, unfortunately, did not produce any useful dosage effects.

Another weakness lay in the impossibility to apply progeny tests to the offspring of the amphiploids because of their sterility. Thus, most tests were made by crossing with New World recessives of the genome formula 2(AD). The offspring raised from 4n(*G. arboreum* × *herbaceum*) × New World tester were therefore AA(AD), those from 4n(*G. anomalum* × *herbaceum*) × New World tester AB(AD), etc.; i.e., they were chromosomally unbalanced. Classification had to be based on phenotypic appearance alone. However, it was felt that such a procedure was sound since the markers employed had very characteristic effects and had been thoroughly studied before (with the exception of the problematical "reduced plant features" from *G. anomalum*).

Old World amphiploids.—The amphiploids of the two closely related species *G. arboreum* and *G. herbaceum* segregated like autotetraploids, giving gametic ratios not wider than 5:1. In fact, in three of five cases the ratio of gametes carrying one or two dominant alleles to gametes with two recessives was rather exactly 4:1 (table 1). This is the opposite of what would have been caused by preferential associations which tend to widen ratios rather than make them narrower. Alternatively, chromatid segregation might have occurred since the number of quadrivalents per cell was rather high (s. below). However, the column headed "plant body" in table 1 in which three classes could be distinguished is rather instructive. The Green class was larger than one fifth of the total, while the Red class was much smaller. One might suppose that elimination rather than chromatid segregation was at play, at least in this case, because the latter would have equally increased the two classes produced from *RR* and *rr*† gametes at the expense of the heterogametic *Rr* class. In contrast, chromosome loss at meiosis in *RRrr* tetraploids would have increased the number of gametes carrying no *R* at the expense of *Rr* gametes and also the number of gametes with one *R* from *RR* gametes producing a shift to types with less or no anthocyanin. On the other hand, loss of *r* could probably not have been detected.

This argument is weakened, however, by some corollary observations. Elimination of chromosomes is infrequent in Old World tetraploids. SARVELLA (1956) counted microcytes in only 5.3 percent of the pollen tetrads produced by 4n(*G. arboreum* × *herbaceum*) and STEPHENS (1942) found little evidence for the transmission of numerically unbalanced gametes in autotetraploid *G. arboreum*. Using genetical methods, GERSTEL (1956) found that there was only an inconsiderable amount of chromosome elimination in 6n(*G. hirsutum* × *raimondii*) as just mentioned. However, irregular distribution of chromosomes could be more pronounced in 4n(*G. arboreum* × *herbaceum*) than in *G. hirsutum* × *raimondii* hexaploids since the latter had only two thirds as many multivalents as the former and only a fraction of the resulting irregularities might become visible in the form of eliminated microcytes. SARVELLA (1956) gave the multivalent frequency for 4n(*G. arboreum* × *herbaceum*) as 9.4 but for 6n(*G. hirsutum* × *raimondii*) as only 6.2.

A third and perhaps the simplest explanation for the disparity between the *RR* and

† Abbreviated from $R_2^{RGS}R_2^{RGS}$ and $R_2^{OGS}R_2^{OGS}$, etc.

rr classes might be that *RR* gametes have a reduced and the *rr* gametes an increased survival value.

The *G. arboreum* × *herbaceum* amphiploids are of particular interest because their segregation might conceivably throw some light on the question of whether the two parent species evolved by means of gene substitution (HARLAND 1936) or through the accumulation of structural differences in their chromosomes (STEPHENS 1950). The latter author suggested three different genetic tests for the recognition of small chromosomal differences in interspecific hybrids: first, the existence of preferential pairing in synthetic allopolyploids; second, the occurrence of gradual elimination of alleles from the donor species in successive backcrosses to the other parent; and third, a comparison of linkage distances in intra- and interspecific backcrosses.

As shown above, STEPHENS' first test was negative when applied to *G. arboreum* × *herbaceum* amphiploids; their segregation ratios indicated random associations of the genetically marked chromosomes from both species. This, however, is not corroborated by a comparison of multivalent associations of chromosomes in autotetraploids with those found in the amphiploid. For 4n *G. arboreum* STEPHENS (1942) reported 10.0 multivalent associations per pollen mother cell (counting octovalents twice) and BEASLEY's 4n *G. herbaceum* "averaged almost ten" quadrivalents (1942). SARVELLA (1956) found the slightly lower number of 9.4 multivalents in the amphiploids of the two species as mentioned. Such a reduction in multivalent number might not be significant, especially as counted by different students under different conditions. But these circumstances do not apply to IYENGAR'S work who observed averages of 9.7 and 8.4 multivalents in autotetraploids of *G. arboreum* and *G. herbaceum* respectively, and the significantly lower figure of 7.0 in their amphiploid (1944). It is also of interest in this connection that one reciprocal translocation of one long chromosome segment has been detected in *G. arboreum* × *herbaceum* hybrids (GERSTEL 1953).

STEPHENS' second test is concerned with the backcross ratios from interspecific hybrids. It was first applied by him (1949) to a *G. hirsutum* × *barbadense* hybrid with the result that there was appreciable selective elimination of donor parent alleles in backcrosses of the hybrid to both parental species. He concluded that the chromosomes of the two species differed; i.e., there was cryptic structural differentiation, to use STEBBINS' (1945) term.

This test was also applied to our material. The results of backcrosses to *G. arboreum* of two different diploid *G. arboreum* × *herbaceum* hybrids contrasted at the *L*, R_2, V_a and *Ne* loci were presented in table 3a. Unfortunately backcrosses to *G. herbaceum*, the other parent species, have not yet been made, and while reciprocal crosses were performed with *G. arboreum* a large majority of the progeny plants came from crosses with the hybrid as pollen parent.

No significant deviation from a 1:1 ratio could be observed for *L*, R_2 and *Ne*. The class with a yellow (*Y*) corolla derived from *G. herbaceum* was deficient in the largest family. Deficiency of yellow plants in progenies of *Yy* heterozygotes has been observed previously and HUTCHINSON (1931) thought it likely that the *y* gene is correlated with a greater rate of pollen tube growth of the gametophytes carrying it. His data came from intraspecific *G. arboreum* crosses, and it is therefore improbable that the reduced number of yellows in our backcross *G. arboreum* × F_1 (*G. arboreum*

× *herbaceum*) expressed a lower efficiency of *Y per se*. However, this cannot be tested by backcrossing the F_1 to a white *G. herbaceum* as no white mutants are known in that species.

Lack of discrimination against genes from the nonrecurrent parent is also borne out by the data of table 3b since the occurrence of zero, one, two, three or four *G. herbaceum* alleles fits the frequencies expected from the expansion of the binomial $(\frac{1}{2} + \frac{1}{2})^4$. Preliminary and one-sided as these data are one may conclude that there is no pronounced tendency for an elimination of chromosomes carrying the alleles from *G. herbaceum* in backcrosses of the F_1 hybrid to *G. arboreum*.

With respect to STEPHENS' third test only very scanty information is available which, however, contradicts the results of the other two and merits further close attention. SILOW (1944) studied a single linkage group, *L–Lc*$_1$, in both pure *G. arboreum* and the hybrid with *G. herbaceum* and found a small, but significant, reduction in crossing over in the interspecific hybrid.

In conclusion, the evidence from amphiploid segregation (table 1) indicates that speciation in the cultivated Old World species (assuming they are distinct species) has proceeded without pronounced changes in the homologies of the chromosomes. If we accept STEPHENS' (1949, 1950) explanation that elimination in backcrosses of alleles derived from the donor species is evidence for a structural differentiation of the chromosomes on which they are located, the data in table 3 also suggest that there is little differentiation of this kind in the cultivated Old World species. On the other hand, IYENGAR (1944) and SILOW (1944) noted some small, but significant, differences between intra- and interspecific materials. These observations should restrain one from attributing all evolutionary changes in the Old World species to gene substitution. It is conceivable that reduction in multivalent frequency in an amphidiploid (IYENGAR's data) and reduction in crossover frequency in an interspecific hybrid (those of SILOW) have come about by purely genetic means, but further elucidation of the problem is needed.

Wild American amphiploids.—Amphiploids between two wild species present less favorable material for a genetic study because usually fewer good genetic markers are available than in cultigens. Taxonomic characters are mostly of complex inheritance and not amenable to precise studies of this kind. *G. thurberi* and *G. raimondii* are two species of very different appearance but probably only the leaf shape and petal spot characters represent genetically simple contrasts. Only petal spot segregation could be evaluated in the present study because the hexaploid *G. hirsutum* × *thurberi* tester used segregated itself for leaf shape. The 363:27 (or 13.4:1) segregation which was obtained differed very significantly ($P<.01$) from a tetrasomic ratio and suggested, therefore, a considerable degree of preferential affinity of the chromosomes of the two parent species. This was also borne out by the cytological observations of SARVELLA (1956) who reported the relatively low number of 3.9 multivalents for the same amphiploid plants. Finally, differences in the chromosomal affinities of *G. thurberi* and *G. raimondii* were also indicated by the sizable differences in segregation ratios and multivalent frequencies between 6n(*G. hirsutum* × *raimondii*) and 6n(*G. hirsutum* × *thurberi*) observed in this laboratory (GERSTEL 1956; SARVELLA 1956).

Old World × G. anomalum *amphiploids.*—The genetic stability of these combina-

tions was striking and corresponded approximately to the low average of 1.9 multivalents per pollen mother cell observed by SARVELLA (1956) on the same plants as were used in our genetic studies. Such a strong expression of differential affinity might appear surprising since the A and B genomes of the component species possess considerable homology. This is demonstrated by a fairly high degree of chiasma formation in their diploid hybrid. In two different diploid F_1 hybrids between Old World cottons and *G. anomalum*, GERSTEL (1953) observed that on the average 16.6 or 17.1 chiasmata per pollen mother cell were formed, compared with 22.3 in pure *G. arboreum*. Yet, as FAGERLIND (1937) has pointed out, the amount of differential affinity increases very rapidly as the genomes are differentiated, while metaphase pairing of the diploid hybrids is only slowly affected. A striking example is afforded by the hybrid between *Primula verticillata* and *P. floribunda* where the chromosome differentiation does not suffice to interfere with complete metaphase pairing in meiosis of the diploid, yet the amphiploid exhibits a very high degree of differential affinity of the chromosomes of the two parent species (UPCOTT 1939).

Our series of amphiploids presents an interesting support of this idea. There is no preferential pairing in the tetraploids with four A genomes, a considerable amount of it in the *G. thurberi* × *raimondii* amphiploids which have four D genomes, much more in the 2(AB) amphiploids and intensely homogenetic association in the *G. arboreum* × *thurberi* amphiploids with genomes from two different continents. This rapid increase of preferential pairing may be illustrated by a tabulation of the amounts of metaphase pairing in diploid F_1 hybrids opposite the segregation ratios of their amphiploids. The metaphase data found in table 8 were taken from various authors and are shown together with the segregation ratios accumulated from tables 1, 4, 5, 6, and 7 of this study. A comparison of the first two rows shows that the segregation ratio of the amphiploids provides a more sensitive measurement of chromosomal non-homologies than the meiosis of the F_1 hybrid. In both *G. arboreum* × *herbaceum* and *G. thurberi* × *raimondii* hybrids sufficiently long homologous segments are present in all the paired chromosomes to permit highly regular bivalent formation, yet in the amphiploid from the second of these hybrids a considerable deviation from the genetic random 5:1 ratio occurs.

An additional point may be raised in connection with the Old World × *G. anomalum* amphiploids; namely, the similarity of segregation values for the different loci which is expressed by the low heterogeneity chi square at the base of tables 5

TABLE 8

Comparison of metaphase affinities of diploid hybrids with segregation ratios of their amphiploids

Hybrid	Mean no. of chromosome associations* per cell in diploid hybrids	Testcross ratios of amphiploids
G. arboreum × *herbaceum*	12.9 GERSTEL (1953)	4.1:1
G. thurberi × *raimondii*	12.9 (BOZA BARDUCCI and MADOO 1941)	13.5:1
Old World × *G. anomalum*	11.6 (GERSTEL 1953)	76.5:1
G. arboreum × *thurberi*	1.0 (WEBBER 1939) 8.1 (SKOVSTED 1937)	0.0:0+

* Quadrivalents counted twice.

and 6. If preferential pairing is due to an accumulation of structural differences in the chromosomes one might expect each set† of chromosomes to have their characteristic degree of preferential pairing because the occurrence of structural changes is a discontinuous process. Thus, one might expect certain *G. anomalum* chromosomes to be still like their Old World homologues and others to be quite changed and to find an expression of this in different amphiploid segregation ratios. Yet, the segregation ratios for factors located on perhaps six different chromosomes were not distinguishable. However, statistical tests involving a 76.5:1 ratio are very obtuse and further investigation on this point with more suitable material is desired before a discussion of its implications. The generality of the phenomenon may also be doubted in view of contrary experiences of GRANT (1954) with 4n *Gilia millefoliata* × *achillaefolia*.

4n(G. arboreum × thurberi).—Almost complete stability is reached in this "typical" amphiploid. Only a single plant differed from the otherwise very uniform progenies (table 7). Some segregation, or irregular chromosome distribution, is expected even in this material with greatly differentiated genomes since multivalent configurations have been observed in its meiosis (BEASLEY 1942).

SUMMARY

Genetical segregation ratios of a series of synthetic allotetraploids were found to widen rapidly with decreasing cytotaxonomic affinity of the component species.

There was no recognizable tendency of the chromosomes of *G. arboreum* × *herbaceum* to associate preferentially; i.e., the gametic ratios were 5:1 or even narrower. This could be interpreted as meaning that the chromosomes of *G. arboreum* and *G. herbaceum* have retained a high degree of homology. Yet, certain cases are quoted from the literature which suggested a certain amount of chromosome repatterning. The amphiploids did not produce functional homogenic gametes (*RR* and *rr* as opposed to *Rr* gametes) with equal frequency and the implications were discussed.

Amphiploids synthesized from the wild American species *G. thurberi* and *G. raimondii* of very different morphology and origin, but both with D genomes, gave an approximate 13:1 backcross ratio for the one locus studied. The difference in segregation ratios between the 4A and the 4D amphiploids shows the greater sensitivity of amphiploid segregation over F_1 pairing to small differences in chromosome homology; pairing of chromosomes at the metaphase of meiosis is normal in both *G. arboreum* × *herbaceum* and *G. thurberi* × *raimondii*.

Amphiploids containing the A and B genomes, both from the Old World and with high F_1 affinity, gave very wide ratios whereas the 2(AD) amphiploid type was almost completely stable.

Segregating amphiploids are suitable for the discovery of linkage relationships; from 4n *G. arboreum* × *herbaceum* it was determined that P_a, and R_2 are independent

† The term "set" has of late been used to designate genomes but was originally applied by BELLING (1921) to a group of two homologues in a diploid or three homologues of a triploid, etc. This older use will be revived here with a slight extension to designate the homologous and partially homologous chromosomes which can replace each other in the gametes of an amphiploid.

and from Old World × *G. anomalum* amphiploids P_a, R_2, Y_a, L and a characteristic called "reduced plant features" segregated independently of each other.

Every amphiploid gave roughly similar segregation ratios for genes located on different chromosomes, as far as could be determined.

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A COMPARATIVE STUDY OF CHROMOSOMAL VARIATION IN SPECIES OF THE MESOPHRAGMATICA GROUP OF DROSOPHILA

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THE adaptive importance of chromosomal polymorphism in Drosophila has been demonstrated through analysis of natural populations as well as through experiments in the laboratory (*cf.* DOBZHANSKY 1951; PATTERSON and STONE 1952; DA CUNHA 1955). However, in different species the genetic variability takes different forms, and chromosomal inversions are characteristic of the adaptive polymorphism in some species of Drosophila but not in others. Even within the groups of species which exhibit variations in gene order, some of them have more inversions than others. A comparative study of closely related members included in various species groups of Drosophila has permitted DOBZHANSKY *et al.* (1950) and DA CUNHA *et al.* (1953) to establish very interesting relationships between the degree of chromosomal polymorphism and the capacity of a species to exploit the ecological opportunities offered by the environment. The greater the ecological versatility of a population or a species, the greater the chromosomal polymorphism, and vice versa.

Nevertheless some very widely distributed and well adapted species exhibit only few inversions or they have none at all (*cf.* PATTERSON and STONE 1952). This is obviously due to the fact that each species has its own means of adjusting itself to the environment. Variations in gene arrangements are not the only index for the adaptive capacity of a species. Each species has its own genetic system which not always can be compared to those exhibited by others.

For an understanding of the origin of such contrasting types of chromosomal adaptive mechanisms, one might logically study the chromosomal structure of different closely related species of which something is known about their evolutionary relationships. In the present paper a preliminary report of the contrasting types of chromosomal variation exhibited by six members included in the *mesophragmatica* group of species of Drosophila is given.

MATERIAL AND METHODS

Six neotropical species of the subgenus Drosophila have been included by BRNCIC and KOREF (1957a, b) in the *mesophragmatica* group: *D. mesophragmatica*, DUDA 1927; *D. gaucha*, JAEGER and SALZANO 1953; *D. pavani*, BRNCIC 1957; *D. altiplanica*, BRNCIC and KOREF 1957; *D. orkui*, BRNCIC and KOREF 1957; and *D. viracochi*, BRNCIC and KOREF 1957. With the exceptions of *D. gaucha* and *D. pavani* which exhibit only cryptic morphological differences, all other members can be distinguished by their external characteristics. There is good chromosomal and genic evidence to show that no gene exchange takes place among the six species of the group.

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Although the geographic distribution areas of the species are by no means completely known, the records indicate that the most widely distributed one is *D. gaucha*, which has been found in southern Brazil, Bolivia, Uruguay and Argentina. *D. pavani* lives only in the north-central part of Chile and in a zone of Argentina close to the eastern slope of the Andes. *D. mesophragmatica* has been found in Bolivia and Peru (Cuzco). *D. altiplanica* is described only from Bolivia. Finally *D. orkui* and *D. viracochi* have been collected only in Peru (Cuzco and Machu Pichu).

According to BRNCIC and KOREF (1957a, b), all six species have five pairs of chromosomes. The metaphase plates of *D. gaucha*, *D. pavani* and *D. viracochi* show one pair of V's, three pairs of rods and a pair of dots. In *D. mesophragmatica* and *D. orkui* the dots were transformed by addition of heterochromatine into a pair of rods. In *D. altiplanica*, one of the pair of rods has acquired the shape of a medium sized V, probably due to the presence of a secondary constriction. In all members of the group, one pair of rods correspond to the sex chromosomes.

In contast to what is observed in the metaphase plates, the structure of the salivary gland chromosomes is very similar in all members of the group. There are five long euchromatic strands and one dot-like chromosome. The disc patterns are sufficiently similar in the homologous chromosomes of the six species under consideration so that the chromosomes can be easily identified. For practical purposes, in the present paper reference to chromosomal variations in the members of the *mesophragmatica* group relate to the "Standard" salivary gland chromosomal map of *D. pavani* published by BRNCIC (1957b).

The material used to investigate the chromosomal polymorphism in the species under consideration was of two kinds: first, a direct analysis of the larval salivary gland cells in the F_1 offspring of females inseminated in nature; and second, the study of genetically heterogeneous stocks of the various species of the group which have been maintained in the laboratory for a few generations. The salivary glands of mature larvae produced in the cultures were stained with the usual aceto-orcein rapid squash method.

OBSERVATIONS

Drosophila pavani

D. pavani have been found in Chile and in Argentina along the eastern slope of the Andes. Up to now, this species has not been collected north of Copiapo, Chile (27° 20′ S.), or south of Valdivia, Chile (39° 52′ S.), or east of San Luis, Argentina (about 66° W.), where it overlaps *D. gaucha*. In the North-central part of Chile, *D. pavani* constitutes the most abundant species of the genus and in different populations its frequency among the genus, ranges between 7.85 percent and 75.43 percent.

The chromosomal polymorphism of this species has been studied in most detail. In a previous report from this laboratory (BRNCIC 1957b) various local populations of this fly had been tested by examination of the gene orders in their chromosomes. Inversions were found in the second and fourth chromosomes. In the second chromosome two inversions were observed, one of them included in the other. This arrangement, A+B, was present in practically all Chilean and Argentinian populations, but the two inversions have never been found separately. The frequency of this

arrangement in heterozygous condition ranges between 28.00 and 56.52 percent in the different populations.

In the right arm of the fourth chromosome all samples analyzed have had three overlapping inversions which form the complex A+B+C gene arrangement. In none of the populations or crosses between them were these three inversions separated. The frequency of heterozygotes for this gene sequence ranges between 46.77 percent and 59.64 percent in the different populations.

In the left arm of the fourth chromosome, in most of the samples studied, there was a complicated gene arrangement which differs from "Standard" by three overlapping inversions. As in the above mentioned case, the three inversions have never been found isolated. In the different populations analyzed, the frequency of heterozygotes for this arrangement ranges between 37.71 percent and 62.28 percent.

Drosophila gaucha

D. gaucha seems to be the most widely distributed species of the group. It has been found in southern Brazil, Uruguay, Argentina and Bolivia. According to personal communications from PROFESSOR C. PAVAN of the University of São Paulo, Brazil, and from PROFESSOR A. CORDEIRO of the University of Rio Grande do Sul, Brazil, *D. gaucha* is a very rare species in tropical Brazil. In Argentina it seems to be more abundant. In the "Sierra" of Cordoba (Argentina) it makes up 31.8 percent and in the "Sierra" of San Luis (Argentina) 16.8 percent of the specimens of the genus.

Owing to the kindness of PROFESSORS PAVAN and CORDEIRO it was possible to analyze strains of *D. gaucha* from São Paulo and Rio Grande do Sul, Brazil. In addition, we have examined the salivary gland chromosomes of the F_1 offspring of wild females inseminated in nature and collected near La Paz, Bolivia (50 females), Cordoba, Argentina (73 females), and San Luis, Argentina (87 females). Neither in the stocks from Brazil nor in the Bolivian and Argentinian populations were found variations in the gene order due to inversions. The F_1 hybrids between the different stocks of *D. gaucha* also showed no inversions.

Drosophila mesophragmatica

The data on the distribution and frequency of *D. mesophragmatica* are somewhat scanty. We have enough information to be assured that the species is abundant in Bolivia and in Peru. In some collecting spots near Cuzco, Peru, the three species, *D. mesophragmatica*, *D. orkui* and *D. viracochi*, make up 51.35 percent to 95.23 percent of the total Drosophila population. Unfortunately, we have no data on the relative frequencies of the three members of the group.

In order to investigate variations in gene order, we have examined stocks of *D. mesophragmatica* from Cuzco (Peru), Machu Pichu (Peru) and La Paz (Bolivia). All the stocks were genetically very heterogeneous since they were obtained from the offspring of more than 20 females caught at each locality. Many larvae were tested in each stock by examination of the salivary gland chromosomes.

In all three populations inversions were found in the third chromosome and in the left arm of the fourth chromosome.

Inversion "A" in the third chromosome. This is a small inversion located in the middle part of the third chromosome (fig. 1). It was found in heterozygous state in about ten percent of the larvae examined from the stocks of La Paz (Bolivia) and Machu Pichu (Peru), and in 20 percent of the larvae from the stock of Cuzco (Peru).

"A+B+C" arrangement in the left arm of the fourth chromosome. This gene sequence is located in the distal half of the left limb of the fourth chromosome, and most probably is constituted by three small inversions whose relationships are difficult to analyse due to the fact that there are many points of nonspecific attractions in this part of the chromosome (fig. 1). The A+B+C arrangement in heterozygous condition was found in more than 50 percent of all larvae examined from La Paz (Bolivia) and Peru (Cuzco and Machu Pichu). None of these populations showed any of the three inversions separate from the others.

In addition to direct analysis of the stocks, F_1 hybrid larvae from the crosses of the three populations under consideration were tested for inversions, but no other variations in the gene orders were found.

Drosophila orkui

D. orkui is sympatric with *D. mesophragmatica* and *D. viracochi* in Cuzco (Peru), and, as mentioned above, the three species are the dominant group in most collecting spots of that place. According to a personal communication from PROFESSOR M. WHEELER of the University of Texas, specimens very similar to *D. orkui* have been collected by DR. W. B. HEED in the mountains near Bogota, Colombia. Our data on chromosomal polymorphism in this specie are very scarce. Only 47 females collected at Cuzco (Peru) and inseminated in nature were tested by examination of the salivary

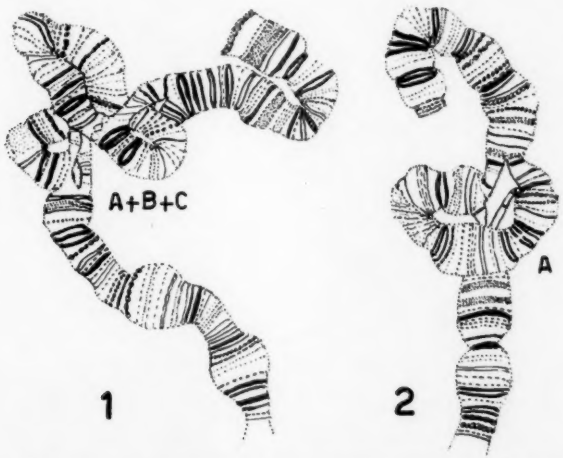

FIGURE 1.—Heterozygous inversions found in *D. mesophragmatica*. 1. Left arm of the fourth chromosomes. 2. Distal part of the third chromosome. The large capital letters designate the inversions described in the text.

gland chromosomes in their progeny. In *D. orkui* three inversions were found in the second chromosome and two inversions in the third (fig. 2).

Inversions in the second chromosome. In the middle of the second chromosome there is a medium size inversion (inversion "A"). Included in the same region is another inversion (inversion "B"), and in the distal half of the chromosome there exists a very small inversion (inversion "C"). Heterozygotes for the "A+B" arrangement were found in 23 out of 47 individuals. Heterozygotes for the "B" inversion alone, occurred in 4 out of 47 larvae, and heterozygotes for the "C" inversion in 12 out of 47 larvae.

Gene arrangements in the third chromosome. In the basal half of the third chromosome there are two small independent inversions. The most basal was named "A", and the most distal, inversion "B". Heterozygotes for the "A" arrangement were found in 4 out of 47 individuals, and for the "B" inversions in 8 out of 47 larvae.

Drosophila altiplanica *and* Drosophila viracochi

D. altiplanica was found only near La Paz (Bolivia) but, according to the samples collected there, it seems to be a very abundant species. Dr. W. B. Heed has col lected some flies very similar to *D. altiplanica* in Colombia, near Bogota, at abou

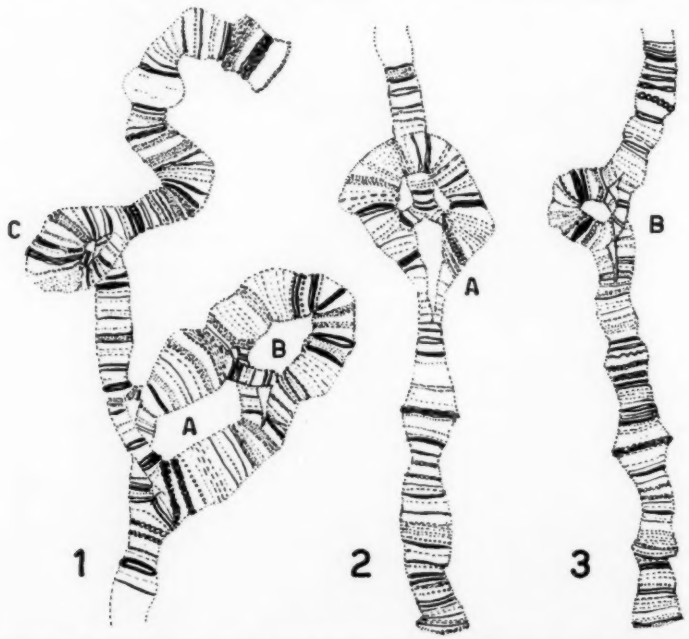

Figure 2.—Heterozygous inversions found in *D. orkui*. 1. The distal part of the second chromosome. 2. and 3. Basal part of the third chromosome. The large capital letters designate the inversions described in the text.

11,000 feet of altitude (personal communication from PROFESSOR M. WHEELER). *D. viracochi* has been collected only in Peru (Cuzco and Machu Pichu).

The offspring of 56 females of *D. altiplanica* from Bolivia, inseminated in nature, were examined in order to investigate variations in their gene order. No inversions were found in them.

The data on chromosomal polymorphism in *D. viracochi* are very scarce. We have examined only three stocks of this fly, one obtained from about a dozen specimens (males and females) collected at Machu Pichu (Peru) by MRS. M. BREUER of the University of São Paulo, Brazil, another stock from the same locality and originated from about five females collected by the author, and finally a third stock obtained from about 20 females caught at Cuzco (Peru). A large number of larvae were tested from each stock, but no variations in the gene order have been observed. Neither were inversions found in the F_1 hybrid larvae obtained in crosses between the three stocks.

DISCUSSION

In table 1 are summarized the numbers of variations in the gene arrangements found in the different members of the *mesophragmatica* group. *D. pavani* has eight inversions, *D. orkui* exhibits five, and *D. mesophragmatica* has only four. In the other three species no variations have been found in the gene order due to inversions. It can be seen at a glance that except for the first chromosome (the sexual pair), inversions have been found in all the other large chromosomes. In *D. pavani* the inversions are concentrated in the second and in both arms of the fourth chromosome; *D. mesophragmatica* are polymorphic with respect to the gene order in their third and in the left arm of the fourth chromosome; *D. orkui* has inversions in the second and third chromosomes.

Though we are far from knowing the exact areas of geographic distribution of the members of the group, there are some data which show that all gene sequences occur very widely throughout the known ranges of the species. This is especially clear in *D. pavani* according to BRNCIC (1957b), and in *D. mesophragmatica* which according to the data here reported, shows no evidence of geographical differentiation in the distribution of inversions.

TABLE 1

Comparison of the numbers of inversions found in the chromosomes of the members of the mesophragmatica *group of species*

Species	Chromosomes					Total
	I(X)	II	III	IV-R	IV-L	
D. pavani	—	2	—	3	3	8
D. gaucha	—	—	—	—	—	0
D. mesophragmatica	—	—	1	—	3	4
D. orkui	—	3	2	—	—	5
D. altiplanica	—	—	—	—	—	0
D. viracochi	—	—	—	—	—	0
Total	0	5	3	3	6	17

It seems interesting to compare this picture with that exhibited by other species of Drosophila. *D. pseudoobscura* (*cf.* DOBZHANSKY 1951), *D. robusta* (CARSON and STALKER 1947), and *D. willistoni* (DA CUNHA *et al.* 1950; DA CUNHA and DOBZHANSKY 1954) are outstanding examples of species in which the gene arrangements show quantitative and qualitative geographic variations. Those three well studied species have very wide geographic areas of distribution. Contrasting with them, the six members of the *mesophragmatica* group have a more restricted range. *D. pavani* lives in the north-central part of Chile and in Argentina close to the eastern slope of the Andes. *D. gaucha* is abundant only in Argentina and seems to be scarce in southern Brazil and Bolivia. *D. mesophragmatica* seems to live only in the high territories of Peru and Bolivia. *D. altiplanica* was found only in Bolivia. Finally, *D. orkui* and *D. viracochi* have been collected only in Peru (Cuzco and Machu Pichu).

Hypothetically a more restricted species is ecologically less versatile, and according to DOBZHANSKY *et al.* (1950) and DA CUNHA *et al.* (1953) would be less polymorphic in comparison to more wide-ranging ones. The high frequency of heterozygotes for inversions that are present in all the range of these species probably depend on the quality of the habitats rather than on their geographical areas. In this connection it will be useful to remember that some of the domestic species of Drosophila, adjusted to the ecological conditions of human life, possess some inversions distributed all over the world.

With the exception of *D. gaucha*, most of the species of the group seem to be fundamentally Andean. The configuration of the Andes mountains leads to the formation of local isolated populations, and this permits rapid speciation. The members of the *mesophragmatica* group can be taken as an example of such a situation, since they are in general allopatric. In contrast with them, widely distributed species in a territory which offers no serious geographic barriers respond to the environmental differences by a high degree of polymorphism which shows gradual clinal variations. This is the case in species such as *D. willistoni* or *D. robusta*. Strongly isolated populations conduce to the formation of many species. It is probable that this has been happening in the evolutionary history of the *mesophragmatica* group. This conclusion emerges from the study of the chromosomal polymorphism exhibited by its members.

BRNCIC and KOREF (1957b) have reported that changes related to speciation in this group involve many paracentric inversions in all pairs of chromosomes. This means, that in the evolutive history of the group all the chromosomes have in the past given rise to variations in the gene order due to inversions. On the other hand, we have not found the intermediate sequences needed to explain most of the complex overlapping inversions present in the members of the group. These facts permit us to conclude that the chromosomal variability actually present in the species of this group is only a fraction of the chromosomal polymorphism which has occurred in the past, and that it represents the last step of a long evolutionary history.

The chromosomal structure of a species is the result of a continuous process of natural selection. The common ancestors of the six members of the *mesophragmatica* group, being transformed in isolated endemic species, have acquired by the selective pressures the gene arrangements best adjusted to each region.

SUMMARY

Several natural populations of the six species included in the *mesophragmatica* group of Drosophila were examined for variations in their gene arrangements. *D. pavani*, *D. mesophragmatica* and *D. orkui* proved to be heterozygous for several inversions each. *D. gaucha*, *D. altiplanica* and *D. viracochi* exhibit no polymorphism due to inversions.

Most of the inversions found in the members of the group are distributed over the entire geographic range of the species. This may be due to the fact that most species are ecologically restricted to isolated places in the Andean system of mountains. The chromosomal polymorphism exhibited by the species of the *mesophragmatica* group represents only a fraction of that which has occurred in the evolutionary history of the group. This may be ascribed to the selective pressures which have preserved only the better adapted gene arrangements in each region.

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CORRIGENDA

VOLUME 41

Page 507, line 11:
for see table 4 *read* see table 3.

VOLUME 42

Page 200, fourth paragraph, second sentence:
for fig. 1 *read* fig. 11
Page 203, first line of legend:
for FIGURES 10–18.—*read* FIGURE 10.—The C chromosome etc.
Page 495, formula following third line of text should read:

$$\left[\frac{a}{2^r}\right]^v \left[(1-a)+\frac{a}{2}\left(1-\frac{1}{2^r}\right)\right]^\beta \left[\frac{a}{2}\left(1-\frac{1}{2^r}\right)\right]^{N-v-\beta}.$$

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